SOME RELATIONS OF PLANTS TO DISTILLED WATER AND CERTAIN DILUTE TOXIC SOLUTIONS

M. C. MERRILL

Formerly Research Assistant to the Missouri Botanical Garden

I. Introduction

In view of the extensive use of distilled water as a medium in which to grow control plants for comparative purposes in solution-culture work, there is well-grounded justification for the performance of considerable experimental work in order to determine more definitely the relations of plants to this medium. The subject is an important one, and it will require much experimentation for the ultimate solution of all phases of the problem involved. While the results herewith reported are only preliminary in their nature, the fact that they give positive indications along certain lines has been deemed sufficient warrant for their publication at this time. In addition to determining the growth relations of plants in this and other media, consideration has also been given to the effect produced by growing plants in this medium as determined by means of electrical conductivity measurements.

II. Historical Aspects of the Subject

The relation of plants to distilled water is a matter that has been under more or less serious consideration at different periods for a long time. Woodward (1699), who first employed the method of water culture in 1691–1692 in his interesting experiments, found that plants grew better in river water than in either rain water, spring water, or distilled water. The difference was of course due to the quantity of plant food contained in the medium, and this idea, coupled also with the character of the nutrients, has been the basis for a vast amount of physiological work since that time.

Coming down to more modern times, there has been a diversity of opinion among the investigators of the subject in regard to the reason why plants and animals thrive so much better in natural water or aqueous media than in distilled water. Considering the period from about 1860 on down to the present, the most important explanations offered may be summed up under the following three heads:

1. Lack of essential nutrients;
2. The presence of deleterious substances;
3. Extraction of salts, or nutrient materials, from the organism immersed in the distilled water.

Holding each of these views there has been a formidable array of scientists at different periods, each group contending strongly to establish the correctness of its viewpoint.

Among the earlier workers in the field may be mentioned Boehm ('75), Dehérain ('78), and others, who believed that the lack of essential nutrients in the distilled water was responsible for the resulting poor condition of the organism. Boehm, for example, believed that calcium played a fundamental rôle in the metabolism of the plant, and that in its absence certain processes, notably that of starch formation, could not be carried on and that therefore deterioration resulted. He also believed that calcium was necessary for the transfer of the reserve materials from the cotyledons to the formative organs. Dehérain repeated Boehm's experiments and confirmed his results.

Owing to the fact that even distilled water, which had been unquestioningly regarded as pure, produced effects simulating toxicity, a great deal of attention has been given in the past to the chemical and other properties of water distilled from different kinds of apparatus and under various conditions. On the animal side, workers, among whom may be mentioned Kölliker ('56) and Nasse ('69), had early noticed the injurious effects on tissues when the same were placed in distilled water. Nasse, for example, found the deleterious effect of distilled water about equal to that of the following solutions: 2.5 per cent $NaCl$, 3.3 per cent $NaBr$, 3.7 per cent Na_2SO_4, and 5.0 per cent NaI.

Nägeli ('93), in his classical work published twelve years after his death, found that very minute amounts of toxic substances, notably copper, in solution produced injurious effects on organisms (*Spirogyra*), and to this phenomenon he applied the term "oligodynamik" action. This line of work was extended to include other substances and other organisms, and claimed the attention at different times of Aschoff ('90), Loew ('91), Locke ('95), Ringer ('97), Copeland and Kahlenberg ('99), Dehérain and Demoussy ('01), Lyon ('04), Bokorny ('05), Hoyt ('13), and others. It is of particular interest to note that Ringer in some of his earlier work ascribed the injury to the extraction from the organism of necessary nutrient materials; but after the publication of Locke's experiments ('95), which Ringer duplicated and confirmed, the latter concluded that the injury done in the particular case under consideration (*Tubifex*) was due to deleterious materials in the distilled water. He says: "Copper in even infinitesimal quantities will disintegrate tubifex whilst water free from copper or other heavy metals and without any salts such as calcium salts can sustain the life of tubifex."

In regard to the third idea pertaining to the effects of distilled water on organisms, early workers, both on the plant and animal side, found that salts were extracted from organisms placed in distilled water, even though their methods for determining the extraction were somewhat crude. Among the early investigators on the animal side may be mentioned Plateau ('83), Ringer and his school ('83, '84, '85, '94, '94^a, '94^b, '97), Loeb ('03), and others. The writer has another paper ready for publication in which is given a historical treatment of the subject of excretions from roots and other plant parts, so the discussion of certain phases of the plant work is reserved for that publication.

Upon the perfection and the employment of conductivity apparatus by physical chemists, it soon began to be used also by the various workers in the fields of soil, plant, and animal investigations. In this connection distilled water came in for its share of consideration. The determination of the purity of water by ascertaining its electrical conductivity speedily came into vogue, and it should be said that as far as electrolytes are concerned it is a very accurate and excellent method and has deservedly come into more and more general use for this purpose in the fields of chemistry, physics, and biology.

Koeppe ('98), for instance, determined the electrical conductivity of water obtained from various sources and compared his results with those of other workers. He believed that distilled water has a deleterious effect which is partly due to a withdrawal of salts necessary to the organism, and partly to a swelling of the tissues. He was supported in his views by Oldham ('09), while Winckler ('04), Kobert ('05), and others argued in favor of the harmlessness of distilled water, especially in medical practice. Peters ('04) used the electrolytic conductivity method in his work on *Stentor* and found that there was an exosmosis of electrolytes when the organism was placed in distilled water, and he therefore concluded that the injurious effects noted were due to an extraction of salts. True and Bartlett ('12, '15, '15^a) considered, for certain salts, not only the excretion but also the absorption of electrolytes under balanced and unbalanced conditions of the medium.

In a recent paper in which a historical discussion of the subject is also given, True ('14) concludes that over and above any injurious effects caused by deleterious substances in the distilled water there is still a "residuum of harmful action due to no known type of impurity." Because this harmful action seems to be most marked in water of least conductivity True believes that the withdrawal of electrolytes from the root tissues best accounts for the deleterious action, but that this withdrawal is "not due to the aggregate difference in osmotic pressure between the cells of the roots and the external medium." He chose lupine seedlings for his work because Frank ('88) had found them very sensitive to distilled water. Schulze ('91), however, after several years of experience with *Lupinus luteus,* claimed that distilled water produced no toxic effects upon those plants.

Both before and after the appearance of the recent contribution by True just referred to, I carried on the investigations reported in this paper, which, as previously stated, are but preliminary in their nature, but which have given indications leading to the conception of an idea differing somewhat from the majority of those above mentioned regarding the relation between plants and distilled water. This conception will be briefly mentioned here, while the evidence and a further discussion will be given later; it is that pure distilled water is not harmful or injurious *per se,* but that because of the static condition forced upon them as a consequence of the absence of plant food, the growing cells become disorganized and thus become easy prey to bacterial and fungous action. Excretion of electrolytes does occur but this should be considered merely as a concomitant condition, or resulting effect of the conditions under which the plants are placed, and should not be considered as a *cause* of degeneration unless the electrolytes themselves be toxic.

III. Methods

(Germination, Culture, and Conductivity)

Canada field peas (*Pisum sativum*) and horse beans (*Vicia faba*), the small variety, were the plants selected, as both were known to be well adapted for growth in solution cultures. Of the various methods of seed sterilization tried out, the one in which the seeds were treated with 1–600 formalin-water for 15 minutes after being soaked for 24 hours in running water gave best satisfaction.

For germinating the seeds a modification of the method used by Boussingault ('74), and also by various investigators in the Bureau of Soils, was employed. This consisted in the use of ordinary enameled-ware pans about 12 inches in diameter and 3 inches in height, filled with tap water and covered with 6 × 6-mesh galvanized iron "hardware cloth," on which the previously soaked and sterilized seeds were placed. The seeds were then covered with filter paper or paper towelling which was kept moist throughout the germination process or until the radicles reached the water below. The germination was carried on in the greenhouse. In the course of four or five days a splendid lot of vigorous, uniform seedlings which have serviceably straight radicles about 2 inches long with no laterals yet formed is obtained by this method; such seedlings are well adapted, both by their character and their accommodation to an aqueous medium, for solution-culture work. At this stage the plumules have grown to about one-half inch in length, and the plants are now ready for transfer to the culture medium, an operation which is easily and quickly done. This method of germination, which is shown in pl. 16 fig. 2, recommends itself both by reason of its simplicity and ease of operation and the certainty of securing excellent results. In the transfer process from the germinating pan to the culture medium, the entire seedling was always immersed and carefully rinsed in once-distilled and again in twice-distilled water; by this means the roots became free of any adhering impurities.

As containers for the cultures, ordinary glass tumblers were used, the sides of which were covered with black paper to prevent algal growth and the top covered with perforated paraffin paper. (For a complete description and illustration of the method see the paper by McCool, '13.) Ten plants were grown in most cases in each tumbler; exceptions to that number will be noted in each case when the series are discussed in detail. Galvanized iron wire supports were used to hold the plants upright when the seedlings had attained sufficient size to require them.

In all cases doubly distilled water was used, the second distillation being carried out in the laboratory with $KMnO_4$ added to the once-distilled water to oxidize any organic matter that might be present. Conductivity tests of this water showed it to possess a specific conductivity of 2.064×10^{-6}. The nutrient solution used was that of Pfeffer, redistilled water being the solvent for the necessary salts. Each tumbler was filled to a convenient level with either the water or the full nutrient solution as the case might be, approximately 250 cc. being required. To replace transpiration loss, doubly distilled water was added as needed.

In the early days of conductivity work on solutions, measurements could be made only by means of a continuous current. Because of the resulting polarization effects, however, the resistance of the solution increased to such an extent as to introduce serious errors into the results. But thanks to the classical work of Kohlrausch and others, the alternating current method was devised and perfected, whereby the determinations became practically independent of polarization effects. A vast amount of work has since been done in the realm of physical chemistry on conductivity measurements, a review of which, however, is outside the scope of this paper. For a clear and concise discussion of this subject see Jones ('09), Walker ('10), or Findlay ('10).

In addition to the investigations already cited which deal with the practical applications of conductivity work, there might well be mentioned in this connection the work done by investigators in the Bureau of Soils of the U. S. Department of Agriculture: Whitney, Gardner, and Briggs ('97); Whitney and Briggs ('97); Whitney and Means ('97); and Gardner ('98). Heald ('02) used the Kohlrausch method for determining the conductivity of plant juices in order to get indications regarding the dissolved mineral substances in different parts of the plants under experimentation. Nicolosi-Roncati ('07), Bouyoucos ('12), Dixon and Atkins ('13, '13[a]) and others have also carried on conductivity determinations with different plants and under various conditions.

Sjöqvist ('95) was the first to use the conductivity method in enzyme investigations, which he did in his work on the action of pepsin on protein solutions. Similar work was done by Oker-Blom ('02), who also extended the applications of this method. Oker-Blom ('12) has recently given an account of his own and previous investigations in the field of bacteriology, wherein the electrical conductivity method was used. Various other investigators have also made use of it, among whom may be mentioned Bayliss ('07). Stiles and Jörgensen ('14) give a partial review of some of the historical aspects of this subject as it pertains to plant work.

For the conductivity work herein reported the following apparatus was used:

Wheatstone bridge (Central Scientific Co., catalogue number, 2475);
Resistance box, 11,110 ohms (Central Scientific Co., catalogue number, 2444);
Induction coil (Eimer and Amend, catalogue number, 4100);
Dry battery cells (Eimer and Amend, catalogue number, 592);
Conductivity cell, Freas (Eimer and Amend, catalogue number, 5202);
Telephone receiver (Central Scientific Co., catalogue number, 2355);
Thermometer graduated to 1/10°C;
Water tank holding 50 gallons, specially constructed for the purpose, pilot flame underneath; temperature regulated to 1/10°C;
Tiffany laboratory motor with which to operate a stirring apparatus in the tank.

In the method employed for the work the procedure given below was consistently followed: the tumblers were always filled to approximately the 250 cc. level with either the solution or redistilled water, depending on the culture. Before taking readings, doubly distilled water was added to bring the water or solution up to the original level, if the transpiration loss since the previous reading made the addition necessary. This was of course essential in order to keep the concentration factor under control. Readings in all cases were taken at 25°C. The control of temperature exactly to within 1/10°C. was comparatively easy by the use of the pilot flame underneath and the stirring apparatus in the tank of water.

For absolutely accurate and final quantitative determinations or ultimate values, as were required, for example, in the case of the standardization measurements for the cell constant with N/50 KCl, or the determination of the specific conductivity of the doubly distilled water, the greatest precautions possible were taken in regard to the conductivity cell and the concentration of its contents. But in making hundreds and even thousands of determinations, most of them as rapidly as accuracy permitted, due to the time factor involved, it was both impossible and unnecessary to dry the cell after each reading, since relative, and not absolute, values were desired for the most part. The method employed, therefore, was to remove from the carefully stirred solution in the tumbler a 25 cc. sample with a pipette of the same capacity, the latter having previously been rinsed with the solution. Using exactly the same amount for each determination further reduced any possibility of error due to unequal dilution in the conductivity cell. Between readings the pipette was kept almost entirely immersed in redistilled water in a tall cylinder attached to a stand in the water bath. After carefully pouring the sample back into the tumbler, in case further readings were to be taken, the cell was rinsed twice with doubly distilled water and rapidly drained before taking the next reading, whether of the same or of a different culture. Any minute amount of doubly distilled water that might be present to dilute the next sample was a constant factor throughout all the readings and was of course inconsequential.

In using fresh batteries it was necessary to insert resistance coils between the battery and the induction coil in order to reduce the current. For this purpose German silver wire was used. While polarization phenomena may possibly be operative to a certain extent, such would be so small as to be practically negligible, especially in view of the fact that the effects from such a cause would be entirely relative and would therefore not affect the validity of the results.

Some of the conductivity results given in this paper are shown in tabular form and others are plotted as curves. In some instances the data are calculated as specific conductivity; in other cases the conductivity is represented by the value of x on the Wheatstone bridge. To make it clear what x actually represents, when the apparatus is set up as it was for the determinations, the following proportion is given:

$$R : R' :: x : 100-x$$

R is the resistance in ohms inserted in the resistance box; R′ is the resistance in ohms of the solution; and x is the number on the bridge wire (graduated in millimeters from 0 to 100 centimeters). As the position of x on the bridge varies with R and R′, the R for each series of curves or tables will be given (though in the great majority of cases it was 9,110), from which R′ can then be calculated. Having these values, the specific conductivity can be calculated for any determined cell constant (the value of the cell used being .4088). For a fuller discussion see Findlay ('10).

IV. Recovery of Plants after being in Distilled Water for Varying Periods

The first question studied pertained to the recovery of plants in full nutrient solution after being kept in doubly distilled water for varying periods. To determine the comparative condition for optimum recovery, the distilled water and the full nutrient medium were renewed every four days in some cultures and left unrenewed in others, in such a way that for either condition of the medium of each set in a certain period the other medium would be both renewed and unrenewed so as to give all possible methods of combination. Examination of table i will make this clear. Thus, for example, with cultures 11, 12, 13, and 14 of the 10-day period in distilled water the doubly distilled water in Nos. 11 and 12 was unrenewed; but when these cultures were placed in full nutrient solution this medium was unchanged or unrenewed for No. 11 and was renewed for No. 12. The distilled water in Nos. 13 and 14 was renewed, and the full nutrient solution unrenewed and renewed respectively.

In series 1 the small variety of horse beans (*Vicia faba*) was employed, 8 plants being used to a culture. The condition of the media and duration of growth, the green weight of tops, and the dry weight of tops and roots of series 1 are given in table i. On examining this table it is seen that even after the plants had remained for 20 days in distilled water, they recovered on being placed in the full nutrient solution, while those remaining for 10 days in distilled water produced practically as much growth when later placed in the full nutrient solution as did the plants which were in the latter medium during the entire period.

Of course, as would be expected, the cultures wherein the full nutrient solution was renewed every four days gave much better growth than did those in the unrenewed medium, due, no doubt, to an increased amount of available nutrients. But an interesting comparison is manifest in connection with the effect of renewing the distilled water; the greater growth of both tops and roots may be noted in cultures 3 and 4, in which the distilled water was renewed every four days throughout the period, as compared with that of cultures 1 and 2, where the distilled water was not renewed, except, of course, for the occasional addition of water to replace the transpiration loss, which, however, was small. Furthermore, in noting the growth of cultures 11–22 inclusive, it is seen that in four of the six cases of comparison between the renewed and unrenewed distilled water, better growth of both tops and roots resulted where the distilled water was renewed. Considering cultures 1–4 and 11–22, inclusive, the total green weight of tops for the unrenewed distilled water as compared with the

TABLE I (Series 1)

EFFECT OF RENEWED VS. UNRENEWED MEDIA ON GROWTH OF HORSE BEANS

Culture no.	Length of period in dist. H_2O days	Dist. H_2O renewed or unrenewed	Length of period in full nutr. days	Full nutr. renewed or unrenewed	Green wt. of tops gms.	Dry wt. of tops gms.	Dry wt. of roots gms.
1	45	Unrenewed	....	....	2.15	.777	.124
2	45	Unrenewed	....	....	1.75	.666	.096
3	45	Renewed	....	....	4.40	.887	.272
4	45	Renewed	....	....	4.40	.870	.235
5	1	Unrenewed	44	Unrenewed	16.30	2.069	.485
6	1	Unrenewed	44	Renewed	27.00	3.069	.707
7	2	Unrenewed	43	Unrenewed	15.85	1.994	.429
8	2	Unrenewed	43	Renewed	32.51	3.543	.743
9	5	Unrenewed	40	Unrenewed	18.90	2.315	.463
10	5	Unrenewed	40	Renewed	26.25	2.895	.700
11	10	Unrenewed	35	Unrenewed	13.35	1.623	.382
12	10	Unrenewed	35	Renewed	26.35	2.928	.697
13	10	Renewed	35	Unrenewed	14.90	1.887	.463
14	10	Renewed	35	Renewed	22.90	2.376	.548
15	15	Unrenewed	30	Unrenewed	14.00	1.719	.388
16	15	Unrenewed	30	Renewed	18.05	1.880	.457
17	15	Renewed	30	Unrenewed	15.60	1.807	.417
18	15	Renewed	30	Renewed	21.20	2.403	.642
19	20	Unrenewed	25	Unrenewed	12.51	1.447	.300
20	20	Unrenewed	25	Renewed	11.40	1.247	.328
21	20	Renewed	25	Unrenewed	11.40	1.319	.284
22	20	Renewed	25	Renewed	15.50	1.670	.454
23	....	....	45	Unrenewed	14.60	1.975	.419
24	....	....	45	Unrenewed	14.70	2.044	.443
25	....	....	45	Renewed	27.85	2.925	.690
26	....	....	45	Renewed	27.05	3.025	.764

renewed distilled water, and the same conditions for the dry weight of tops and roots, gave the results to be seen in table II. The total weight in all cases is therefore greater in the cultures in which the distilled water was renewed.

TABLE II (Series 1)
EFFECT OF RENEWED VS. UNRENEWED DISTILLED WATER ON GROWTH OF HORSE BEANS
(Summarized Results of Part of Table I)

Medium	Green wt. of tops in grams	Dry wt. of tops in grams	Dry wt. of roots in grams
Water renewed...	110.30	13.219	3.315
Water unrenewed	99.56	12.287	2.772

These results therefore indicate that the so-called injury to plants in distilled water cannot be entirely or even satisfactorily explained on the basis of extraction of solutes from the plant tissues. If that were the case we should have the greatest injury and least recovery in those cultures in which the distilled water was renewed, the periodically renewed water effecting *in toto* a greater exosmosis of the salts than the water which is not renewed. This statement will receive verification under the section on conductivity measurements. It would therefore seem that we must seek other explanations for the phenomena observed when plants are placed in distilled water. This phase of the subject will also be discussed later.

The points noted will be clear from an examination of pl. 13 figs. 1 and 2. Plate 13 fig. 1 shows the various stages of recovery after varying periods in the distilled water. The better growth is to be noted of both tops and roots of No. 2, in which the distilled water was renewed, as contrasted with No. 1, in which it was not renewed. It is interesting to observe how plants, even after 20 days in distilled water, will recover in full nutrient solution and then give even better growth than plants in unrenewed full nutrient solution the entire period, and that after 10 days in distilled water, plants will recover in renewed full nutrient solution and equal in growth, plants grown the entire period in renewed full nutrient solution.

Plate 13 fig. 2 shows first (Nos. 1 and 2) the contrasted effect of renewing and not renewing the full nutrient solution. The remaining 8 cultures of the plate show the effect of renewing and of not renewing both the distilled water and the full nutrient solution. In cultures 3–10 the comparison should, of course, be made between the alternating numbers for the distilled water effect (renewed or not renewed), and between successive numbers for the effect of the renewal or the non-renewal of the full nutrient solution. While the culture represented by No. 7 of the plate gave greater growth than did No. 9, that excess was probably due to the individual hardihood of two plants. It is seen that a much more uniform and desirable growth was made by the plants of No. 9.

An interesting point in connection with the horse beans is that 16 days after setting up the series the tips of those plants still in distilled water were more or less blackened, probably as a result of enzyme (oxydase) action, and many of them were considerably inrolled. Such conditions were entirely absent from the cultures in full nutrient solution at that time. When the affected plants were later placed in full nutrient solution there was a gradual recovery from the blackening of the leaves, and this recovery was greater in the case of those cultures in which the distilled water had been renewed than in those in which it had not been renewed. Twenty days later Nos. 3 and 4 were in very much better condition than Nos. 1 and 2. There was much less blackening, some leaves not being blackened at all. The general height of the plants in Nos. 1 and 2 was 1½–2½ inches; and in Nos. 3 and 4 it was 2½–4 inches. A very noticeable feature at the end of the experiment was the condition of the medium, that of Nos. 3 and 4 being of course clear while that of Nos. 1 and 2 was milky, turbid, and opaque, indicating abundant fungous and bacterial action, a condition further emphasized by the hyphal threads and gelatinous coating on the roots.

The roots of the plants in Nos. 3 and 4 were also in much better condition at the end of the experiment than were those of Nos. 1 and 2, especially as regards length and the amount of lateral root development. The root growth in No. 13 at the end of the experiment was also greater than that in No. 11; but in Nos. 12 and 14 it was about equal. The plants of No. 17 also showed greater root growth than did those of No. 15, and this difference was more marked than in the case of the tops. The lateral roots in No. 17 were produced all along the main roots, while in No. 15 they were practically confined to the upper or older portion of the main roots. Another interesting difference observed was that in No. 17 the main root tips were not permanently injured in the distilled water and when placed in the full nutrient solution they continued growth. This was not the case in No. 15. In general there was not much difference between the roots in Nos. 16 and 18; the plants in No. 18, however, had slightly greater growth of roots and showed less injury and some continuation of growth of the tips, whereas those in No. 16 did not. The same condition of the roots above noted for Nos. 15 and 17 held also in Nos. 19 and 21 respectively; but the difference in favor of the renewal of the distilled water though less marked was nevertheless evident. Likewise, Nos. 18 and 20 were similar to Nos. 16 and 18 respectively.

Strong evidence was therefore afforded by the cultures of horse beans that renewing the distilled water has a favorable effect upon the plants.

Series 2 is in every respect a duplicate of series 1 except that Canada field peas (*Pisum sativum*) were used instead of horse beans (*Vicia faba*), and that the dry weight of the tops was not determined; furthermore, the length of the experimental period was different. The condition of the media and duration of growth, the green weight of tops, and the dry weight of roots are given in table III, seven plants being grown in each culture. An examination of this table reveals results similar in many cases to those contained in table I; plants recovered even after 20 days in distilled water, but after 10 days in this medium the recovery was not so complete as in the case of the horse beans, for the plants so treated did not equal in growth similar ones which had remained in full nutrient solution the entire period. However, plants which had been in distilled water only 5 days before being transferred to full nutrient solution subsequently equalled in growth other plants which had been in the latter medium from the start. The period between 5 and 10 days in distilled water is therefore a critical one, and will be discussed later in other connections.

TABLE III (Series 2)

EFFECT OF RENEWED VS. UNRENEWED MEDIA ON GROWTH OF PEAS

Culture no.	Length of period in dist. H_2O days	Dist. H_2O renewed or unrenewed	Length of period in full nutr. days	Full nutr. renewed or unrenewed	Green wt. of tops gms.	Dry wt. of roots gms.
1	47	Unrenewed	....	....	.80	.073
2	47	Unrenewed	....	....	.60	.076
3	47	Renewed	....	....	.45	.067
4	47	Renewed	....	....	1.30	.091
5	1	Unrenewed	32	Unrenewed	6.75	.400
6	1	Unrenewed	32	Renewed	11.05	.500
7	2	Unrenewed	31	Unrenewed	5.50	.401
8	2	Unrenewed	31	Renewed	12.90	.568
9	5	Unrenewed	28	Unrenewed	5.95	.263
10	5	Unrenewed	28	Renewed	12.35	.467
11	10	Unrenewed	23	Unrenewed	5.35	.254
12	10	Unrenewed	23	Renewed	7.90	.321
13	10	Renewed	23	Unrenewed	3.80	.160
14	10	Renewed	23	Renewed	6.07	.202
15	15	Unrenewed	18	Unrenewed	3.30	.144
16	15	Unrenewed	18	Renewed	4.40	.175
17	15	Renewed	18	Unrenewed	4.30	.162
18	15	Renewed	18	Renewed	4.15	.169
19	20	Unrenewed	13	Unrenewed	3.21	.124
20	20	Unrenewed	13	Renewed	2.52	.092
21	20	Renewed	13	Unrenewed	4.05	.139
22	20	Renewed	13	Renewed	4.43	.141
23	....	....	33	Unrenewed	5.50	.368
24	....	....	33	Unrenewed	6.94	.420
25	....	....	33	Renewed	9.60	.536
26	....	....	33	Renewed	13.45	.611

Renewing the full nutrient solution again showed beneficial results, as might be expected. But the renewal of the distilled water did not produce such striking results in some respects as in the case of the horse beans; in other ways, however, the results were equally or even more striking. Where the plants remained in distilled water for 47 days the growth was better in one case and poorer in the other where the distilled water was renewed than where it was not renewed. The average growth, however, of the two cultures in the renewed medium was better than that of the two in the unrenewed distilled water.

In Nos. 11–22, there was better growth of tops and roots in four cases where the distilled water was renewed and better growth in four cases where it was not renewed. Considering cultures 1–4 and 11–22 the results given in table IV were obtained, from which it is again evident that renewing the distilled water exercises no injurious influence, and the conclusion is reinforced that an exosmosis of mineral nutrients is not the fundamental basis of the injury which plants suffer in distilled water. Furthermore, the difference between the renewed and the unrenewed distilled water cultures was very marked if the plants remained for 20 days in distilled water before being changed to the full nutrient solution, the difference being greatly in favor of the cultures in which the medium was renewed.

TABLE IV (Series 2)

EFFECT OF RENEWED VS. UNRENEWED DISTILLED WATER ON GROWTH OF PEAS

(Summarized Results of Part of Table III)

Medium	Green wt. of tops in grams	Dry wt. of roots in grams
Distilled water renewed . . .	28.55	1.131
Distilled water unrenewed.	28.08	1.259

Figures 1 and 2 of pl. 14 illustrate the points above mentioned. In pl. 14 fig. 2 should be noted the better growth of Nos. 9 and 10—which were in renewed distilled water for 20 days before transfer to full nutrient solution—as compared with Nos. 7 and 8, which had remained in unrenewed distilled water for the same length of time before transfer. The excess of growth in No. 4 over that in No. 6 is probably to be accounted for on the ground that since those cultures were in distilled water but 10 days neither the renewal nor the unrenewal of the medium exercised much effect. Hence the greater growth of No. 4 represents an individual variation.

At the expiration of the experimental period the following conditions prevailed in series 2: while the top growth in cultures 1–4 was about the same in each case, the root growth in Nos. 3 and 4 was much better than that in Nos. 1 and 2, the roots of the former being whiter, cleaner, and having longer and more numerous lateral roots. In the case of those cultures grown in distilled water 10 days before removal to full nutrient solution, Nos. 11 and 12 were in somewhat better condition than Nos. 13 and 14, a difference which might readily be expected for the shorter periods in distilled water due to individual variation. After 15 days in distilled water and 18 days in full nutrient solution the benefits derived from renewing the former were markedly evident in the appearance of cultures 15–18, even though the actual weights did not show such difference. Nos. 17 and 18 were in better condition than Nos. 15 and 16 respectively, especially as regards the root growth; similarly, Nos. 21 and 22 were in better condition than Nos. 19 and 20 respectively.

Some special conditions which are of particular interest were observed when the cultures were examined carefully at the close of the experiment. The first point pertains to the method of recovery. After being in the distilled water only one or two days the top growth of such cultures when placed in full nutrient solution proceeds unhindered from the tips of the main stems, i. e., the tips of the stems remain uninjured and resume growth. But 5 days in distilled water almost marks the limit at which growth can be resumed at the tip of the main axis of the stem when such cultures are subsequently placed in full nutrient solution. After 10 days in distilled water the tips of the stems become injured so that the later growth in full nutrient solution is made from new lateral branches. Hence the period from 5 to 10 days in distilled water before removal to full nutrient solution may be considered a crucial period as regards the recovery and growth of the main stems.

Another point of interest is the delayed maturity which results in the case of the cultures which are grown for some time in distilled water and later are placed in full nutrient solution. Such plants remain in a green and growing condition much longer than do those which have been in full nutrient solution for the entire period, or those which remained in distilled water for a shorter period before being transferred to the full nutrient solution. The growing season of the former is thus prolonged and the date of maturity delayed.

The foregoing series having given evidence of the recovery of plants in full nutrient solution after being in distilled water for 20 days, the question arose as to the maximum length of time plants might remain in distilled water without preventing recovery when subsequently transferred to full nutrient solution. Series 3 was therefore set up. This consisted of cultures of Canada field peas grown in distilled water for 10, 20, 30, 40, and 50-day periods before transfer to the full nutrient medium. The condition of the media and duration in each and also the results of the series (as shown by the green weight of tops) are given in table v, Nos. 1–20 inclusive. Renewals in this series also were made every four days. Nos. 21–28 under different conditions and concentration of nutrient solution are given for purposes of comparison. The maximum time limit in distilled water above referred to is thus seen to be approximately 30 days, and this was practically attained only in case of the cultures in renewed distilled water. After 40 days in distilled water, whether renewed or unrenewed, the recovery was almost nil, though somewhat better in the renewed, while after 50 days in either renewed or unrenewed distilled water all the cultures were dead.

In the 10 cases furnishing comparisons between cultures in which the full nutrient solution was preceded on the one hand by renewed and on the other by unrenewed distilled water, greater growth was attained in 7 cases where the distilled water was renewed. The total weight of green tops is more nearly equal in the two sets of cultures, however, being 24.20 grams in the case of those in the unrenewed and 22.38 grams in the case of those in the renewed distilled water. We thus see that no injurious effects attend the renewal of the distilled water when compared with the non-renewal of the same; on the other hand, positive benefits are derived from such a renewal, especially in the case of plants approaching the maximum time limit of durability in distilled water—a period which enables the results of the two conditions to be more readily seen and compared.

TABLE V (Series 3)

GROWTH OF PEAS IN RENEWED AND UNRENEWED MEDIA FOR VARIOUS PERIODS UP TO THE MAXIMUM TIME FOR SURVIVAL. ALSO EFFECT OF ADDING WATER AT DIFFERENT INTERVALS TO MEDIA UNDER VARIOUS CONDITIONS

Culture no.	Length of period in dist. H_2O days	Dist. H_2O renewed or unrenewed	Length of period in full nutr. days	Full nutr. renewed or unrenewed	Green wt. of tops gms.
1	10	Unrenewed	42	Unrenewed	4.50
2	10	Unrenewed	42	Renewed	8.00
3	10	Renewed	42	Unrenewed	4.85
4	10	Renewed	42	Renewed	6.30
5	20	Unrenewed	32	Unrenewed	2.70
6	20	Unrenewed	32	Renewed	5.05
7	20	Renewed	32	Unrenewed	3.55
8	20	Renewed	32	Renewed	1.40
9	30	Unrenewed	22	Unrenewed	.55
10	30	Unrenewed	22	Renewed	1.30
11	30	Renewed	22	Unrenewed	1.90
12	30	Renewed	22	Renewed	1.90
13	40	Unrenewed	12	Unrenewed	.50
14	40	Unrenewed	12	Renewed	.55
15	40	Renewed	12	Unrenewed	.65
16	40	Renewed	12	Renewed	.72
17	52	Unrenewed	....	....	.60
18	52	Unrenewed	....	....	.45
19	52	Renewed	....	....	.56
20	52	Renewed	....	....	.55
21	Unrenewed full nutr. 42 days, dist. H_2O added every 8 days				6.40
22	Unrenewed full nutr. 42 days, dist. H_2O added every 4 days				6.00
23	Renewed full nutr. 42 days, the sol'n. renewed every 8 days				8.75
24	Renewed full nutr. 42 days, the sol'n. renewed every 4 days				18.50
25	Unrenewed 1/10 full nutr. 42 days, dist. H_2O added every 4 d'ys				2.90
26	Unrenewed 1/5 full nutr. 42 days, dist. H_2O added every 8 days				2.95
27	Renewed 1/10 full nutr. 42 days, sol'n. renewed every 4 days				10.10
28	Renewed 1/5 full nutr. 42 days, sol'n. renewed every 8 days				7.85

In pl. 15 fig. 1 some of the cultures are illustrated, the ones of special interest being Nos. 9–14. The exceptionally small or irregular growth of No. 8 is difficult to account for, because in the renewed full nutrient it should be greater than that of No. 7. Individual resistance is apparent, however.

V. Recovery of Plants after being in Toxic Solutions

Having thus ascertained the maximum time plants may remain in distilled water and then recover on being placed in full nutrient solution, we may turn our attention to toxic solutions. If distilled water in itself is toxic then it should be interesting to get quantitative data on its effects as measured by the power of plants so treated to recover. This power should furnish a good index regarding the extent of any injury suffered. By comparing the ultimate time limits for various media after which recovery in full nutrient solution is possible, we are able to get a basis on which to determine the relative toxicity of each medium. Almost simultaneously with series 3, series 4 was set up. The plan of the series and the green weight of tops and dry weight of roots of the plants in series 4 are given in table VI, while pl. 15 fig. 2 shows the actual condition of the plants in some of the media. The results obtained indicate the following relative toxicities of the substances used, the time expressed in days having reference to the longest period in the toxic solution after which recovery is possible:

Redistilled water	30–40 days
N/100 $MgCl_2$	4–8 days
N/1000 $MgCl_2$	about 20 days
N/1000 $CaCl_2$ & N/20 $MgCl_2$	about 16 days
N/12800 H_2SO_4	about 20 days
N/400 KOH	about 20 days

We thus see that as compared with the toxic solutions mentioned distilled water, if it be considered as a toxic agent at all, is much less so than either of the others given above. In this connection it is interesting to note that Kahlenberg and True ('96) found that N/12800 H_2SO_4 and N/400 KOH were approximately the critical concentrations for Lupinus roots. Hence, the fact that plants can remain much longer in distilled water than in these solutions and still recover would seem to indicate that as regards toxicity distilled water is only very slightly if at all deleterious. But the writer believes that it is entirely incorrect and misleading to speak of distilled water as being toxic. What is illustrated above for distilled water is not toxicity, therefore, but merely the length of time

TABLE VI (Series 4)

EFFECT ON GROWTH OF PLANTS OF VARIOUS PERIODS IN TOXIC SOLUTIONS

Culture no.	First sol'n. or medium	Length of period in first medium days	First medium renewed or unrenewed	Length of period in full nutr. days	Green wt. of tops gms.	Dry wt. of roots gms.
1	Dist. H_2O	32	Unrenewed	....	1.15	.116
2	Dist. H_2O	32	Renewed	....	1.55	.130
3	N/100 $MgCl_2$	32	Unrenewed	....	.35	.012
4	N/100 $MgCl_2$	32	Renewed	....	.40	.016
5	N/100 $MgCl_2$	1	Unrenewed	31	10.15	.428
6	N/100 $MgCl_2$	2	Unrenewed	30	8.40	.372
7	N/100 $MgCl_2$	4	Unrenewed	28	5.15	.132
8	N/100 $MgCl_2$	8	Unrenewed	24	.35	.018
9	N/100 $MgCl_2$	12	Unrenewed	20	.30	.020
10	N/100 $MgCl_2$	16	Unrenewed	16	.40	.016
11	N/100 $MgCl_2$	20	Unrenewed	12	.28	.012
12	N/1000 $MgCl_2$	32	Unrenewed	....	1.00	.085
13	N/1000 $MgCl_2$	32	Renewed	....	1.00	.038
14	N/1000 $MgCl_2$	2	Unrenewed	30	8.85	.385
15	N/1000 $MgCl_2$	4	Unrenewed	28	9.70	.384
16	N/1000 $MgCl_2$	8	Unrenewed	24	7.20	.305
17	N/1000 $MgCl_2$	12	Unrenewed	20	5.15	.192
18	N/1000 $MgCl_2$	16	Unrenewed	16	2.05	.121
19	N/1000 $MgCl_2$	20	Unrenewed	12	1.05	.093
20	N/1000 $CaCl_2$ and N/20 $MgCl_2$	32	Unrenewed	....	.75	.092
21	N/1000 $CaCl_2$ and N/20 $MgCl_2$	32	Renewed	....	.85	.099
22	N/1000 $CaCl_2$ and N/20 $MgCl_2$	1	Unrenewed	31	10.60	.409
23	N/1000 $CaCl_2$ and N/20 $MgCl_2$	2	Unrenewed	30	9.35	.388
24	N/1000 $CaCl_2$ and N/20 $MgCl_2$	4	Unrenewed	28	10.35	.384
25	N/1000 $CaCl_2$ and N/20 $MgCl_2$	8	Unrenewed	24	8.40	.294
26	N/1000 $CaCl_2$ and N/20 $MgCl_2$	12	Unrenewed	20	3.00	.144
27	N/1000 $CaCl_2$ and N/20 $MgCl_2$	16	Unrenewed	16	1.50	.117
28	N/1000 $CaCl_2$ and N/20 $MgCl_2$	20	Unrenewed	12	.75	.103
29	Full nutr. sol'n.	32	Unrenewed	32	8.95	.411
30	Full nutr. sol'n.	32	Renewed	32	18.50	.530
31	N/12800 H_2SO_4	32	Unrenewed	....	1.55	.130
32	N/12800 H_2SO_4	32	Renewed	....	1.25	.124
33	N/12800 H_2SO_4	2	Unrenewed	30	7.05	.318
34	N/12800 H_2SO_4	8	Unrenewed	24	7.45	.289
35	N/12800 H_2SO_4	16	Unrenewed	16	4.40	.236
36	N/12800 H_2SO_4	20	Unrenewed	12	1.95	.172
37	N/400 KOH	32	Unrenewed	....	1.25	.094
38	N/400 KOH	32	Renewed	....	1.50	.108
39	N/400 KOH	2	Unrenewed	30	8.60	.444
40	N/400 KOH	8	Unrenewed	24	6.60	.214
41	N/400 KOH	16	Unrenewed	16	2.55	.092
42	N/400 KOH	20	Unrenewed	12	2.60	.117

plants can survive in a medium without nutrient materials. That these plants could not survive for that length of time in the other media, however, shows that in those cases a real toxicity enters into consideration.

In addition to the actual time limits for recovery just tabulated, as well as the method of recovery and delayed maturity mentioned in the preceding section, another interesting point, which was very noticeable in the cultures and which can also be seen in the plates, is the character of growth of the rootlets in the boundary cultures, by which is meant those cultures which have remained in the inimical media nearly as long as their endurance would permit, and whose recovery in full nutrient solution is slower or more difficult than the normal unaffected plants. In the latter case the roots are short and compact and usually extend down only to about one-half the distance to the bottom of the tumbler. In the case of the first mentioned cultures, however, when transferred to full nutrient solution the rootlets develop a long, slender growth easily extending to the bottom of the tumbler.

VI. Effect of Sterilizing the Water During Growth of Plants

The foregoing series pointed, therefore, to factors other than extraction or loss of solute from the plant tissue as being responsible for the deteriorating phenomenon observed when growing plants are placed in distilled water. In the unrenewed water cultures in the previous series a brownish coloration developed and the roots appeared, in their gelatinized condition, to be covered by bacterial and fungous growths. Suspecting that these organisms played an important rôle, it was decided to grow additional cultures to test this point. Four cultures, each containing ten plants of *Pisum sativum*, were set up in distilled water: in one the medium was not renewed; in a second the water was renewed every four days; and in the remaining two the medium was sterilized every four days by boiling in a return condenser one-half hour. The results are given in table vii (series 5) and the cultures are shown in pl. 16 fig. 1. The full nutrient solution cultures were grown for purposes of comparison. The duration of growth was 30 days.

Whether the beneficial effect of the sterilization was due to the destruction of the bacterial and fungous floras of the medium, to a decomposition of any contained toxic substances (thereby rendering them less toxic), or to incidental effects such as aëration of the water by the boiling process, was not definitely determined. Neither was this effect compared with that produced by the addition of various bodies (tannic acid, pyrogallol, calcium carbonate, various hydrates, carbon black, and other substances mentioned by Livingston and his co-workers, '05, '07, Dachnowski, '08, '09, and others). In the last paper of Livingston and his co-workers referred to are given the results of boiling the aqueous extracts from soils containing toxic properties as determined by the growth of plants in the same. The boiling improved the extracts, but this effect was explained by "supposing the process of boiling to remove or change the toxic action of this extract, the toxic materials being perhaps partly volatile with steam." But since in our sterilization process a return condenser was used the removal of toxic substances by volatilization would not occur. A breaking down of toxic compounds into less toxic constituents may possibly be a condition induced by the boiling, however. It will be recalled that Lyon ('04) found the toxicity of tap water reduced by boiling.

TABLE VII (Series 5)

EFFECT PRODUCED ON GROWTH OF PLANTS BY STERILIZING THE WATER IN WHICH THEY ARE GROWN

Culture no.	Medium	Condition of medium	Green wt. of tops gms.	Dry wt. of roots gms.
1	Dist. H_2O	Unrenewed	1.55	.141
2	Dist. H_2O	Renewed	1.65	.150
3	Dist. H_2O	Sterilized	2.40	.225
4	Dist. H_2O	Sterilized	3.05	.233
5	Full nutr.	Unrenewed	10.30	.342
6	Full nutr.	Renewed	17.65	.507

While the oxidizing power of roots, due to enzymatic activity, may be an important factor in aiding in the decomposition of vegetable matter in the soil, as pointed out by Schreiner and Reed ('07) and others, it is not believed that in the case under consideration the oxidizing power of the roots was altered to any appreciable degree by the boiling of the medium. Dachnowski ('12) mentions the effect of oxidation upon the toxic substances found in bog water. In the sterilization method by boiling under a return condenser, however, the aëration or oxidation phenomenon would no doubt play only a subsidiary rôle. The stronger line of evidence seems to favor the destruction of injurious bacterial and fungous agencies as the chief factor in the beneficial effect of the sterilization.

VII. CONDUCTIVITY MEASUREMENTS

The excellence of the electrical conductivity method for determining any change in the electrolyte content of an aqueous medium naturally led to its adoption for the experimental work described below. This phase of the investigation was especially concerned with determinations pertaining to the extraction of electrolytes — including the essential nutrient salts—from the roots of plants in distilled water. The generally beneficial results attendant upon a frequent renewal of the distilled water in which the plants were placed has already been noted, as well as the evidence in favor of the view that conditions other than extraction of essential salts constitute the underlying cause of the deterioration of plants in distilled water.

The next point to be determined was the relative amount of the total exosmosis in the renewed distilled water as compared with that in the unrenewed. In placing roots in distilled water it is pertinent to this subject to inquire whether all the exosmosis occurs during the first four days. If it does, we should have the same amount of extraction in both the unrenewed water and that renewed every four days. Or is there a renewal of the exosmosis of the electrolytes following the renewal of the water each time, thereby giving rise to a greater exosmosis than in the cultures in which the water was not renewed? If such a condition obtains and yet in spite of it the renewal of the water shows no baneful effects, or indeed produces beneficial results, then may we well conclude, and with increasing assurance, that extraction of nutrient salts is in no way responsible for any injury plants undergo in distilled water. The results obtained strongly substantiate that conclusion.

A series of cultures (series 6) was set up in which healthy plants of Canada field peas were grown in full nutrient solution for about three weeks and then transferred, after carefully rinsing the roots, to doubly distilled water. In half of the cultures the distilled water was renewed at certain definite intervals for each culture, while in the other half of the cultures the water was not renewed. Conductivity determinations were then made of the water under both conditions—renewal and non-renewal—at certain regular intervals, varying for each set of cultures, for several days after the plants had been placed in this medium.

By numerous readings it was ascertained that with a resistance of 9,110 ohms in the resistance box the average value of x on the Wheatstone bridge for the water in the vessel after being rinsed and before placing the roots therein was approximately 6.0, rarely varying 1 cm. either way. Considering that figure, then, as the basis or the starting point for the exosmosis, and subtracting it from the different values found for the renewed, and from only the final value obtained for the unrenewed distilled water, we get the figures in the last column of table VIII.

The plan of the experiment with respect to renewal of the distilled water and the time of readings, the values of the individual readings, and the comparative amounts which represent the total exosmosis of the electrolytes under the various conditions of the experiment are all given in table VIII. The numbers given are the values of x on the Wheatstone bridge when the resistance inserted in the box was 9,110 ohms.

It is thus seen that by far the greater exosmosis was obtained in the case of those cultures in which the distilled water was renewed. Another point of interest was the reabsorption of electrolytes—as seen by the decrease in conductivity of the medium—in those cultures in which the distilled water was not renewed. The reabsorption of electrolytes has been observed to be a phenomenon characteristic of normal, healthy peas, when transferred from a full nutrient solution to distilled water, after being in the latter medium one or two days.

TABLE VIII (Series 6)

COMPARATIVE EXOSMOSIS IN RENEWED AND UNRENEWED DISTILLED WATER

Culture no.	Water renewal	Conductivity Readings								Duration of treatment days	Total increase in conductivity
		Frequency	1st	2nd	3rd	4th	5th	6th	7th		
1	Every day	Every day	32.9	10.4	10.0	8.9	9.7	9.4	10.2	7	49.5
2	None	Every day	36.3	22.8	21.4	17.8	15.2	12.5	11.4	7	5.4
3	Every 2 days	Every 2 days	10.8	9.3	9.6	10.7	....	....	....	8	16.4
4	None	Every 2 days	25.0	14.3	13.6	11.0	....	....	....	8	5.0
5	Every 4 days	Every 4 days	12.9	15.0	16.1	16.1	....	....	....	16	36.1
6	None	Every 4 days	10.7	12.4	15.9	19.5	....	....	....	16	13.5

In order to obtain some additional information regarding the relations between the conductivity of the medium and the plants grown therein, series 7 containing 50 cultures was set up in full nutrient solution, ten Canada field pea plants to each culture. The nutrient solution was not renewed. At the end of each five-day period 5 of the cultures were taken down, the green weight of tops of the plants in each determined, and the conductivity of the solution measured; and from these results the average green weight of tops and the average conductivity of each set of 5 cultures were obtained. This was done throughout the entire period of 50 days. The results obtained are given in table IX and plotted as curves in fig. 1. In the latter the abscissa represents days, and the ordinate both specific conductivity and green weight of tops. The values given for conductivity should be multiplied by 10^{-5}

in order to get the specific conductivity values. In the case of the weights the numbers in the margin represent ten times the actual weight in grams, e.g., 40 in the margin = 4.0 grams.

From the results it is seen that both the increase in green weight of tops and decrease in conductivity of the medium are most rapid and pronounced during the first 15 days. After that period both the green weight and the conductivity gradually decline, but the latter more slowly than the former. While the curves of the minimum, average, and maximum conductivity remain very close together during the first 15 days, they become more divergent after that time. The green-weight curve shows a gradual decline as the age of the plant increases, after a certain period, due to the drying of the tops and consequent loss of water. Different curves would, of course, have been obtained had the nutrient solution been renewed.

TABLE IX (Series 7)

GROWTH OF PLANTS AND CONDUCTIVITY OF FULL NUTRIENT MEDIUM FOR 50 DAYS

Cultures nos.	Length of period in full nutrient days	Av. green wt. of tops in each culture grams	Specific conductivity* at end of period		
			Minimum	Average	Maximum
1- 5	5	3.81	93.22	96.25	98.19
6–10	10	8.12	57.47	61.03	66.93
11–15	15	9.47	32.38	34.83	37.59
16–20	20	8.66	24.38	32.68	46.05
21–25	25	8.69	15.73	18.19	21.69
26–30	30	8.00	13.74	20.63	30.28
31–35	35	7.10	10.02	15.98	21.23
36–40	40	6.77	11.16	14.23	16.19
41–45	46	6.09	6.88	11.44	22.51
46–50	50	5.01	13.00	16.97	28.11

* The numbers in the three columns are to be multiplied by 10^{-5} in order to arrive at the specific conductivity values.

In table x and fig. 2 are seen the results of series 8, a similar experiment with distilled water, the same units being used as in the previous case. The green weight of tops increased during the first 10 days and then gradually declined to the end of the experiment. The conductivity of the water was practically the same on the 10th as it had been on the 5th day. Evidence from other experiments, however, indicates

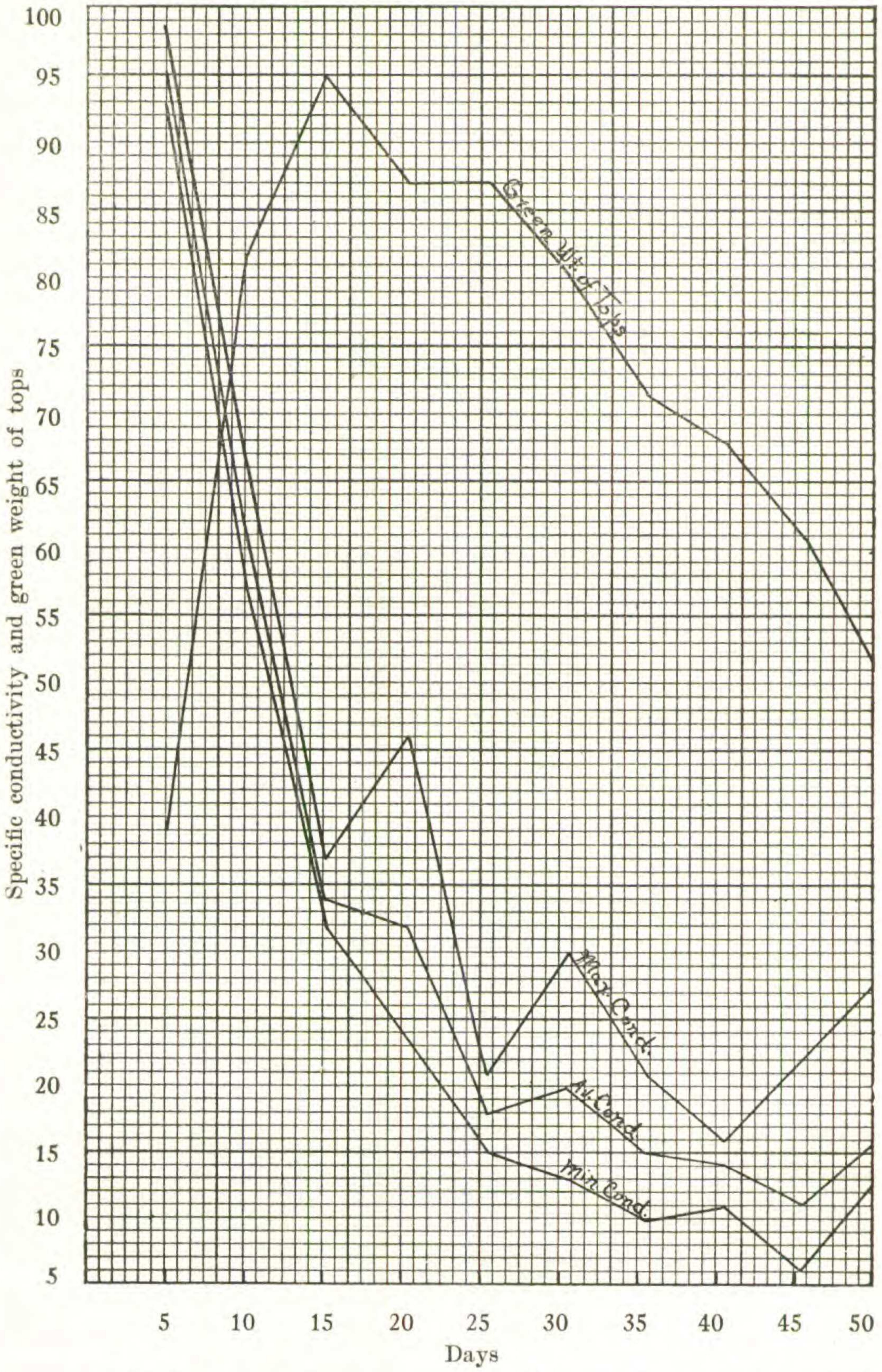

Fig. 1. The conductivity and growth curves for the full nutrient solution (Pfeffer's) in which plants were grown 50 days, the medium being unrenewed. (For complete explanation see the text.)

that in the interim the curve might have risen and fallen. After the 10th day the curve inclined with fluctuations. Here again are seen evidences that the 10-day period for seedlings in the distilled water may properly be considered a crucial one for the plants. After that time the growth declines and the conductivity increases markedly.

Suspecting that the question of injury to plants in distilled water might be intimately bound up with that of lack of reserve food materials, the writer carried out an experiment bearing upon this matter. The experiment consisted, first, in placing some Canada field pea seedlings directly into redistilled water and determining the specific conductivity of the water at intervals for 20 days; and, next, in transferring some pea plants which had been grown in full nutrient solution for 1, 5, 10, 20, 30, and 40 days respectively to redistilled water, and determining the specific conductivity of the water at intervals for 20 days. The results are plotted as curves in fig. 3, the conductivity values being represented in terms of x on the Wheatstone bridge with a resistance of 9,110 ohms in the box. Four cultures of 10 plants each (except in the case

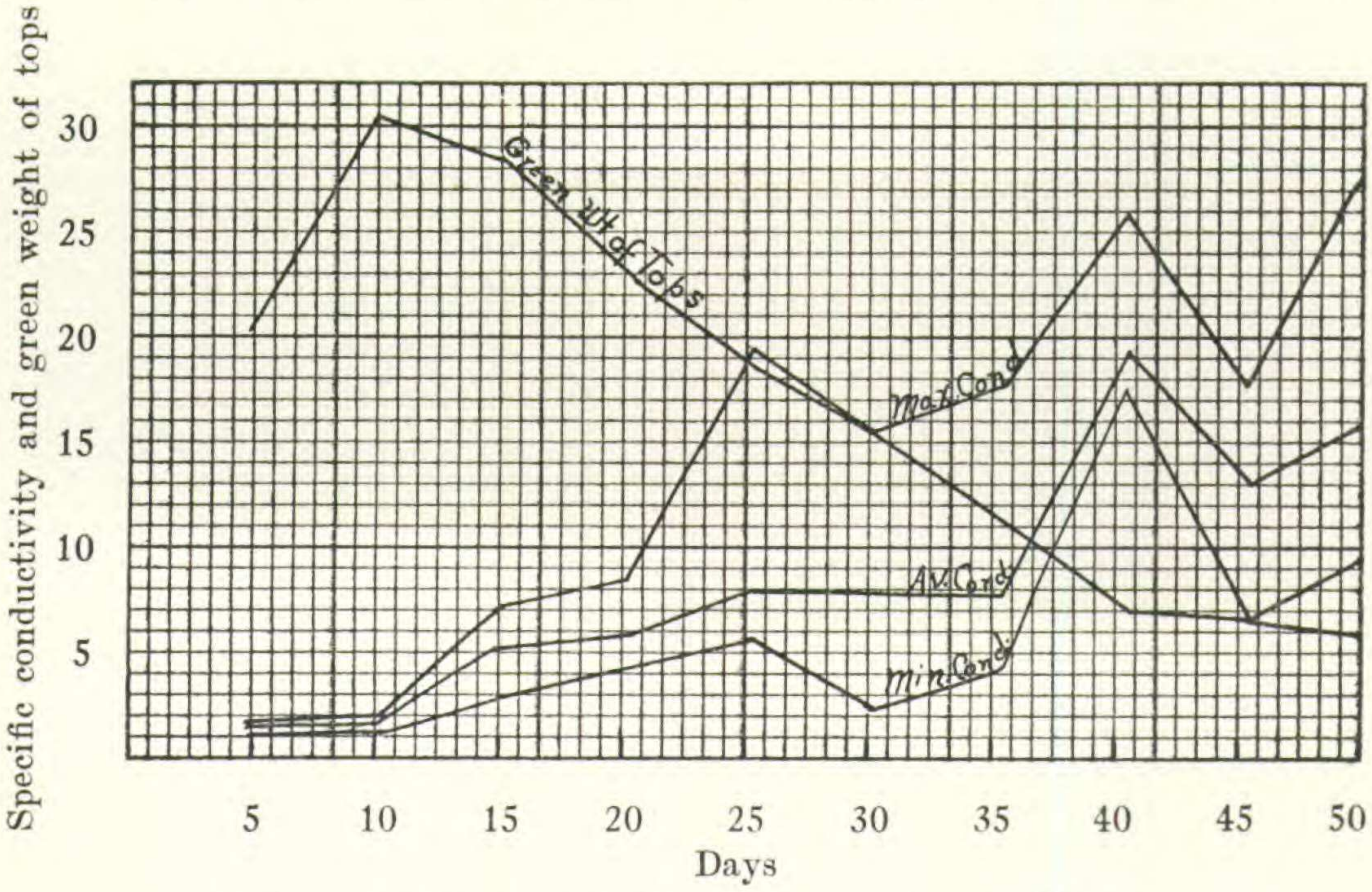

Fig. 2. The conductivity and growth curves for the unrenewed redistilled water in which pea seedlings were grown for 50 days. (For complete explanation see the text.)

of the 20-day period in full nutrient solution in which 12 cultures were used) were grown under each of the specified conditions, and the curves represent the averages for the 4 (or 12) cultures under each condition. To determine how much increase in conductivity was contributed by the glass tumblers in which the cultures were grown, 4 such containers filled only with redistilled water, and containing no plants, were used and the conductivity of the water determined at intervals for 20 days. It is seen that from the seedlings which had not been in full nutrient solution at all (Nos. 5–8) the highest conductivity resulted, while from those which were in the full nutrient solution longest before being placed in the distilled water (Nos. 37–40 and 33–36), the lowest conductivity was found at the end of 20 days. The other cultures at the end of 20 days were midway between the two extremes. It is also seen that, whereas the conductivity curve for Nos. 5–8 shows very little tendency to decline in the early stages, the curves for the cultures which had first been in full nutrient solution show that tendency to a considerable extent. And that tendency, as we have previously remarked, is a characteristic feature of normal plants transferred from full nutrient solution to distilled water.

TABLE X (Series 8)

GROWTH OF PLANTS AND CONDUCTIVITY OF DISTILLED WATER MEDIUM FOR 50 DAYS

Cultures nos.	Length of period in dist. water days	Av. green wt. of tops grams	Specific conductivity* at end of period		
			Minimum	Average	Maximum
1– 5	5	2.04	1.46	1.54	1.66
6–10	10	3.07	1.01	1.34	1.81
11–15	15	2.89	3.04	5.43	7.41
16–20	20	2.34	4.34	5.92	8.63
21–25	25	1.89	5.66	8.15	19.77
26–30	30	1.55	2.60	8.26	15.82
31–35	35	1.15	4.43	7.80	17.95
36–40	40	.72	17.62	19.51	26.04
41–45	45	.68	6.87	13.32	17.95
46–50	50	.60	10.51	16.48	29.00

* The numbers in the three columns are to be multiplied by 10^{-5} in order to arrive at the specific conductivity values.

Attention should be called to the difference in the character of the conductivity curves in fig. 2 and that of 5–8 in fig. 3. It will be noted that both represent the conductivity curve of distilled water containing the roots of seedling peas. The difference mentioned no doubt finds its explanation in the different conditions under which the two series were grown (the series

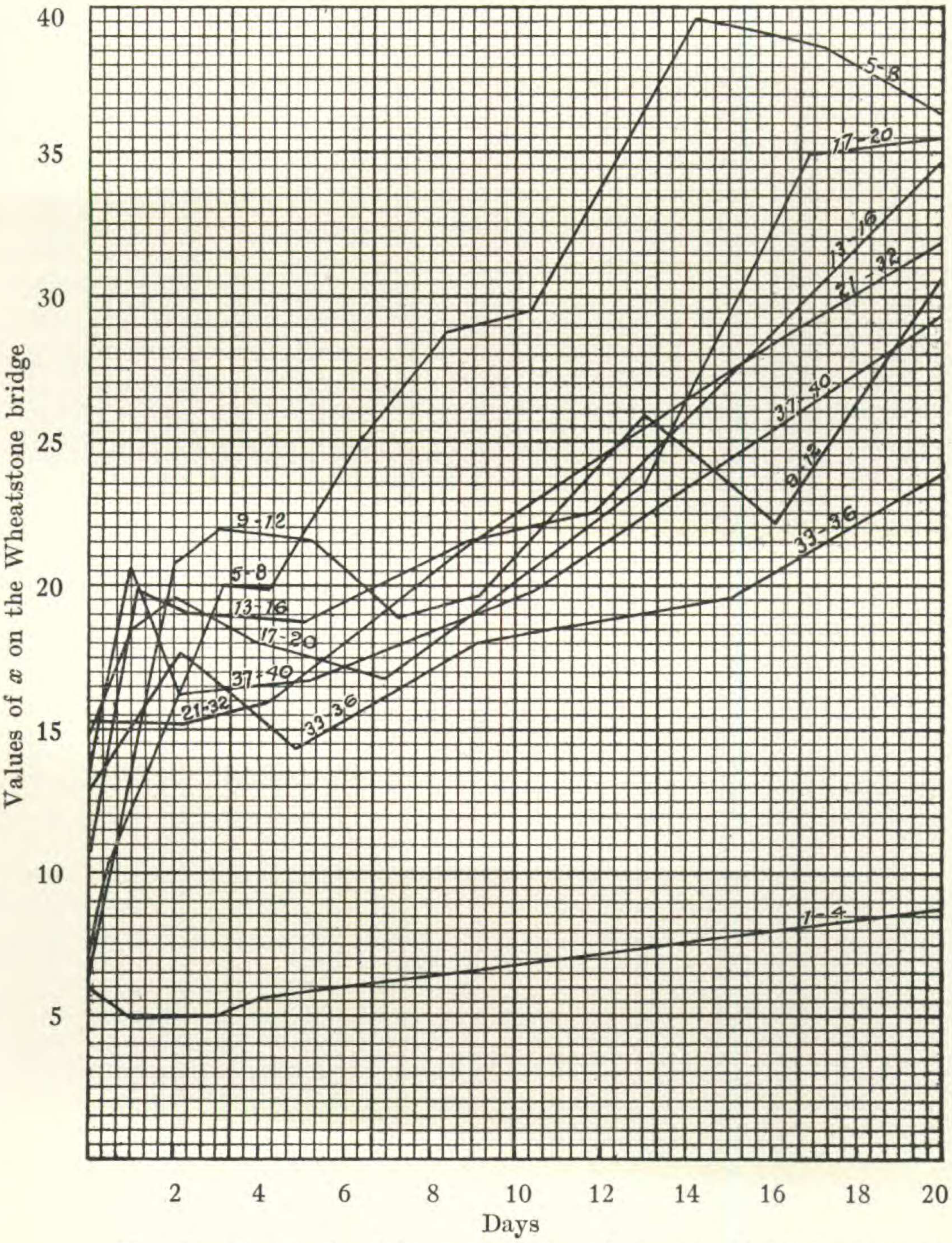

Fig. 3. The conductivity curves for cultures in distilled water 20 days—after growth in full nutrient solution for varying periods of time, as follows: Nos. 9–12, 1 day; Nos. 13–16, 5 days; Nos. 17–20, 10 days; Nos. 21–32, 20 days; Nos. 33–36, 30 days; Nos. 37–40, 40 days. Nos. 5–8 were grown only in distilled water, while Nos. 1–4 were without plants, consisting only of distilled water.

for fig. 2 being run in the fall when the seeds were fresh, and that for fig. 3 in the winter), in the vigor of the seeds, and in the difference in the units used in plotting the curves. It must be said, however, that various factors of the problem of exosmosis from the roots of plants remain as yet unknown.

The early drop in the curve of the conductivity of the controls (1–4) is an interesting feature which would seem to be explained by an adsorption of the electrolytes on the surface of the chemically clean glass tumblers.

At the end of 20 days in distilled water the roots of the plants which had not been in full nutrient at all showed marked deterioration (being badly decomposed and covered with a gelatinous coating), while the roots of those which had previously been in full nutrient solution for some time remained normal in every respect, even after 20 days in distilled water.

These results seem plainly to indicate that injury which plants sustain in distilled water is very closely related either to the lack of available nutrients in the medium or of reserve food material in the tissues. A seedling is in an exceedingly plastic state of growth. If no food materials become available the embryonic tissues which are in such an active condition of growth soon become disorganized, possibly suffering partial autolysis and becoming the prey to bacterial and fungous action. We would expect, therefore, that the larger the seeds (and hence also the supply of stored materials), the longer the seedlings could remain in distilled water before deterioration. Comparison of True's results on *Lupinus* with those here presented on *Pisum sativum* and *Vicia faba* seems to fulfill that expectation. We should also expect that the more nutrient materials the plant absorbed, the better it would be able later to withstand any deteriorating influences in the distilled water, and the experiment above noted seems to bear out that idea also.

In the light of what has been said we are led to believe that the conductivity curve of Nos. 5–8 is not a pure representation of exosmosis and that the products of bacterial and fungous action and cell decomposition account for at least a part of the conductivity. While the same condition may be true of the other cultures to a certain extent, it no doubt plays a lesser, and real exosmosis a greater, part.

In connection with the above experiment it was thought desirable to determine whether a difference in the initial temperature of the water into which the roots were placed had any immediate or subsequent effect upon the exosmosis from the roots; plants which had been grown in full nutrient solution for 20 days were used for this purpose. Four cultures were prepared with distilled water at a temperature of 6.5°C., four at 17.2°C., and four at 35.0°C., and conductivity readings were taken after exactly one-half hour, and then at various intervals for 20 days. No attempt was made to keep the water at the initial temperatures and it therefore gradually returned to the temperature of the room. After one-half hour, when the first readings were taken, the respective temperatures were 8.9°C., 16.6°C., and 27.4°C.

The average conductivities of the water of these cultures are plotted for 20 days in fig. 4, the same units being used as in fig. 3. From these results it may be concluded that the initial differences of temperature can not be said to have exercised much, if any, effect. The results would probably have been different had the temperatures remained at the original point during the 20 days. Wächter ('05) has considered the rôle of the temperature factor in exosmosis.

VIII. Discussion and Conclusions

It is believed that the evidence furnished is sufficient to support the conclusion that pure distilled water *per se* is not toxic or injurious to plants, and that various other factors enter in to cause the deterioration noted when plants are placed in that medium.

Of course by qualifying the assertion to include *pure* distilled water only, we have thus eliminated the effect that may be produced by toxic substances in the distilled water, no matter from whence derived. The abundance of work that has been done on the toxicity of various substances to plant tissues would of course lead us to expect injurious effects if such substances were present in any quantity in the distilled water. With that phase of the question we are therefore not much concerned at present. With a distilled water prepared as indicated, and with a specific conductivity which is approximately 2×10^{-6}, we have a water sufficiently pure for use in the consideration of other aspects of the question, and attention is directed to these.

The evidence presented has inclined us strongly to the view that the fundamental basis of the deterioration of plants in distilled water rests upon the food relations of such plants, but that, on the other hand, an exosmosis of food materials or nutrient salts is in no way responsible for the difficulty. It is considered that the question of the food relation plays an important rôle in the incipiency of the disorder, but that this is quickly followed by factors which have been initiated as a result of the inimical food or nutrient relation.

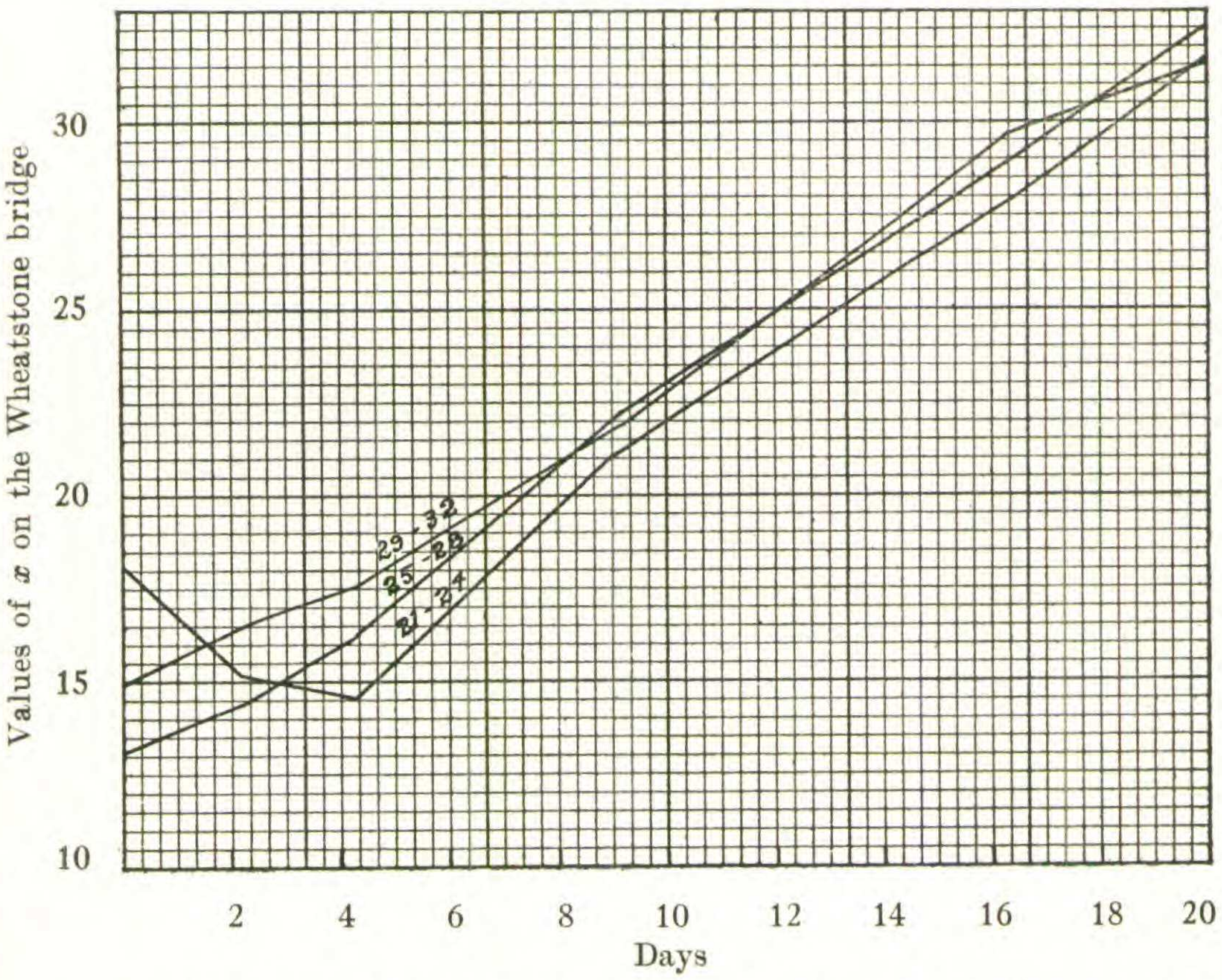

Fig. 4. The conductivity curves for cultures in distilled water 20 days—after growth in full nutrient solution for 20 days. The initial temperatures of the distilled water into which the roots were placed were as follows: Nos. 21–24, 6.5°C.; Nos. 25–28, 17.2°C.; Nos. 29–32, 35°C.

A plant must assuredly have food in order to thrive. The more food it has stored up in its tissues, the longer it can survive in a medium devoid of it. But because of the absence of available food it is believed that the tissues of the plant begin to become disorganized and in that condition fall a ready prey to bacterial and fungous action, which may then set in and play a very important part in the subsequent decomposition of the tissues.

While it may seem paradoxical to assert in one clause that absence of food is the fundamental basis of the injury which plants undergo in distilled water, and in the very next to say that exosmosis of nutrient salts plays no rôle, yet the results obtained have substantiated that idea. Furthermore, it is essential to consider the various other factors attendant upon these two conditions in order to arrive at the proper conclusions respecting their operation. Among such factors may be mentioned the decrease in conductivity after a short period coincident with exosmosis from normal tissues, the relation of sterilization to bacterial and fungous action, the recovery of plants under different conditions, and the numerous other questions already considered in the body of the article, all of which lend weight to the conclusions arrived at.

IX. Summary

A brief historical review is given in this paper of the views held in regard to the cause of injury to plants in distilled water.

The methods of work are outlined.

The experimental work is given and the results discussed, especially with reference to the conclusions of other workers.

A discussion is given of the results obtained in the experimental work and the conclusions derived therefrom are stated.

Some of the results obtained from the experimental work may be summarized as follows:

(a). Renewing the distilled water of the cultures every 4 days was in general beneficial, as shown by increased growth of both tops and roots. The plants were also able to survive longer in the renewed than in the unrenewed distilled water, and continued growth better after being placed in a full nutrient solution.

(b). The period between 5 and 10 days in distilled water is a crucial one for plants; if they remain longer in this medium they are unable to recover normally or completely when subsequently placed in a full nutrient solution.

(c). By keeping the plants in distilled water a certain period before transferring to full nutrient solution the maturity of the plants is delayed.

(d). The longest period during which plants can be kept in distilled water and later recover on being placed in full nutrient solution was found to be 30–40 days. For certain dilute toxic solutions this period was much less, thus indicating that the so-called toxicity of distilled water is, if it exists at all, very slight.

(e). The lateral roots of "boundary cultures" were characteristically long and thread-like.

(f). Sterilizing the distilled water by boiling one-half hour every 4 days exercised a beneficial effect upon the growth of plants in that medium as compared with the growth of those in unsterilized distilled water.

(g). Greater total exosmosis was obtained in the renewed than in the unrenewed distilled water.

(h). Normal plants which have been grown for some time in full nutrient medium and then transferred to distilled water exhibit at first greater excretion than absorption of electrolytes. After one or two days, however, there is greater absorption than excretion and the conductivity curve declines. This condition may be maintained for a considerable period.

(i). The conductivity curve of the full nutrient solution in which plants were grown rapidly fell during the first 15 days or so; then it was more or less horizontal for a period, and finally began to incline after about 50 days. The growth curve was in general opposite in character to the conductivity curve.

(j). The conductivity of the distilled water in one series in which the roots of pea seedlings were placed was practically the same on the 10th as on the 5th day. After the 10th day it rose considerably. The growth curve showed a rise the first ten days, then a decline.

(k). Higher conductivity in the distilled water after 20 days was caused by plants which had not previously been in full nutrient solution than by plants grown for a time in full nutrient solution before transference to distilled water. The former cultures also failed to give the decline in conductivity characteristic of normal plants transferred from full nutrient solution to distilled water.

(l). Greater deterioration of the roots in distilled water occurred if the plants had not previously been in full nutrient solution than in the case of plants which had been grown for a time in the latter medium.

(m). Initial difference of temperature of the distilled water produced no effect on the exosmosis of electrolytes.

The sincere thanks of the writer are cheerfully extended the following, who have generously aided in various ways in the preparation of this paper: Dr. B. M. Duggar, for his helpful suggestions and criticisms throughout the work; Dr. J. F. Merrill and Prof. Lindley Pyle, for suggestions in regard to some features of the conductivity apparatus; Mr. C. H. Thompson, for the photographic work; and Mrs. Amy Lyman Merrill, for assistance in making the calculations, plotting the curves, and in numerous other ways.

Literature Cited

Aschoff, C. ('90). Ueber die Bedeutung des Chlors in der Pflanze. Landw. Jahrb. **19**: 113–141. *pl. 2–4.* 1890. [See p. 115.]

Bayliss, W. M. ('07). Researches on the nature of enzyme-action. I. On the causes of the rise in electrical conductivity under the action of trypsin. Jour. Physiol. **36**: 221–252. 1907.

Boehm, J. ('75). Über den vegetabilischen Nährwerth der Kalksalze. Sitzungsber. d. k. Akad. d. Wiss., Wien, math.- naturw. Cl. **71** : 287–304. 1875.

Bokorny, T. ('05). Das Kupfer und die Giftwirkung des destillierten Wassers. Chemiker-Zeit. **29**: 687–688. 1905.

Boussingault, J. ('74). Sur la rupture de la pellicule des fruits exposés a une pluie continue. Endosmose des feuilles et des racines. Agron., Chim., Agr., et Physiol. **5**: 303–310. 1874. [See pp. 308–310.]

Bouyoucos, G. ('12). Transpiration of wheat seedlings as affected by different densities of a complete nutrient solution in water, sand, and soil cultures. Beih. z. bot. Centralbl. **29**^{1}: 1–20. *f. 3.* 1912. [See pp. 14–15.]

Copeland, E. B., and Kahlenberg, L. ('99). The influence of the presence of pure metals upon plants. Wis. Acad. Sci., Trans. **12**: 454–474. 1899.

Dachnowski, A. ('08). The toxic property of bog water and bog soil. Bot. Gaz. **46**: 130–143. *f. 1–6.* 1908.

————, ('09). Bog toxins and their effect upon soils. *Ibid.* **47**: 389–405. *f. 1–2.* 1909.

————, ('12). Peat deposits of Ohio, their origin, formation, and uses. Ohio Geol. Survey, 4th Ser. Bul. **16**: 1–424. *pl. 1–8. f. 1–29.* 1912. [See pp. 307–342.]

Dehérain, P. P. ('78). Sur l'assimilation des substances minérales par les plantes. Ann. Agron. **4**: 321–349. 1878. [See pp. 334–345.]

————, et Demoussy, E. ('01). Sur la germination dans l'eau distillée. Compt. rend. acad. Paris **132**: 523–527. 1901.

Dixon, H. H., and Atkins, W. R. G. ('13). Osmotic pressures in plants. II. Cryoscopic and conductivity measurements on some vegetable saps. Roy. Dublin Soc., Sci. Proc. N. S. **13**: 434–440. 1913.

————, ————, ('13a). Osmotic pressures in plant-organs. III. The osmotic pressure and electrical conductivity of yeast, beer, and wort. *Ibid.* **14**: 9–12. 1913.

Findlay, A. ('10). Practical physical chemistry. London, 1910. [See pp. 144–181.]

Frank, B. ('88). Untersuchungen über die Ernährung der Pflanze mit Stickstoff und über den Kreislauf desselben in der Landwirthschaft. Landw. Jahrb. **17**: 421–553. *pl. 10–13.* 1888. [See p. 535.]

Gardner, F. D. ('98). The electrical method of moisture determination in soils: results and modifications in 1897. U. S. Dept. Agr., Div. Soils, Bul. 12 : 1–24. *pl. 1–3. f. 1.* 1898.

Heald, F. D. ('02). The electrical conductivity of plant juices. Bot. Gaz. **34**: 81–92. *f. 1–2.* 1902.

Hoyt, W. D. ('13). Some toxic and antitoxic effects in cultures of Spirogyra. Torr. Bot. Club, Bul. **40**: 333–352. 1913.

Jones, H. C. ('09). The elements of physical chemistry. New York, 1909. [See p. 377 ff.]

Kahlenberg, L., and True, R. H. ('96). On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. **22**: 81–124. 1896.

Kobert, R. ('05). Einiges Medizinische über das Wasser. Zeitschr. f. Krankenpflege **27**: 377–384. 1905.

Koeppe, H. ('98). Reines Wasser, seine Giftwirkung und sein Vorkommen in der Natur. Deut. med. Wochenschr. **24**: 624–626. 1898.

Kölliker, A. ('56). Ueber die Vitalität der Nervenröhren der Frösche. Würzburger Verhandl. **7**: 145–147. 1856.

Livingston, B. E., Britton, J. C., and Reid, F. R. ('05). Studies on the properties of an unproductive soil. U. S. Dept. Agr., Bur. Soils, Bul. 28 : 1–39. 1905.

———, Jensen, C. A., Breazeale, J. F., Pember, F. R., and Skinner, J. J. ('07). Further studies on the properties of unproductive soils. *Ibid.* 36 : 1–71. *pl. 1–7.* 1907.

Locke, F. S. ('95). On a supposed action of distilled water as such on certain animal organisms. Jour. Physiol. **18**: 319–331. 1895.

Loeb, J. ('03). On the relative toxicity of distilled water, sugar solutions, and solutions of the various constituents of the sea-water for marine animals. Univ. Cal. Publ., Physiol. **1**: 55–69. 1903.

Loew, O. ('91). Bemerkung über die Giftwirkung des destillirten Wassers. Landw. Jahrb. **20**: 235. 1891.

Lyon, E. P. ('04). A biological examination of distilled water. Marine Biol. Lab., Bul. **6**: 198–202. 1904.

McCool, M. M. ('13). The action of certain nutrient and non-nutrient bases on plant growth. Cornell Univ. Agr. Exp. Sta., Mem. 2: 113–216. *f. 1–15.* 1913.

von Nägeli, C. ('93). Ueber oligodynamische Erscheinungen in lebenden Zellen. Neue Denkschr. d. allgem. schweiz. Ges. f. gesam. Naturwiss. **33**: 1–52. 1893.

Nasse, O. ('69). Beiträge zur Physiologie der contractilen Substanz. Pflüger's Archiv f. gesam. Physiol. d. Menschen u. d. Thiere **2**: 97–121. *1 f.* 1869.

Nicolosi-Roncati, F. ('07). Ricerche su la conduttivita elettrica e la pressione osmotica nei vegetali. Rendic. dell' Accad. Sci. Fis. e Mat. (Sezione della Soc. Reale de Napoli). III^{a} **13** : 357–364. 1907.

Oker-Blom, M. ('02). Die elektrische Leitfähigkeit und die Gefrierpunktserniedrigung als Indicatoren der Eiweissspaltung. Skand. Archiv f. Physiol. **13**: 359–374. 1902.

———, ('12). Die elektrische Leitfähigkeit im Dienste der Bakteriologie. Centralbl. f. Bakt. Abt. I. **65**: 382–389. *f. 1–4.* 1912.

Oldham, R. S. ('09). Is snow-water unwholesome? The Lancet **1909**^{2}: 1240–1241. 1909.

Peters, A. W. ('04). Metabolism and division in Protozoa. Am. Acad., Proc. **39**: 441–516. 1904.

Plateau, F. ('83). Influence de l'eau de mer sur les animaux d'eau douce, et de l'eau douce sur les animaux marins. Compt. rend. acad. Paris **97**: 467–469. 1883.

Ringer, S. ('83). The influence of saline media on fishes. Jour. Physiol. **4**: VI–VIII. 1883. [See section, "Proc. of the Physiol. Soc."]

———, ('84). Concerning the influence of saline media on fish, etc. *Ibid.* **5**: 98–115. 1884.

———, ('97). The action of distilled water on Tubifex. *Ibid.* **22**: XIV–XV. 1897–1898.

———, and Buxton, D. W. ('85). Concerning the action of small quantities of calcium, sodium, and potassium salts upon the vitality and function of contractile tissue and the cuticular cells of fishes. *Ibid.* **6**: 154–161. 1885.

———, and Phear, A. G. ('94^{a}). The influence of saline media on the tadpole. *Ibid.* **17**: 423–432. 1894–1895.

———, ———, ('94^{b}). The influence of saline media on Tubifex Rivulorum. *Ibid.* **17**: XXIII–XXVII. 1894–1895. [See section, "Proc. of the Physiol. Soc."]

————, and Sainsbury, H. ('94). The action of potassium, sodium, and calcium salts on Tubifex Rivulorum. *Ibid.* **16:** 1–9. 1894.

Schreiner, O., and Reed, H. S. ('07). The rôle of the oxidizing power of roots in soil fertility. Jour. Biol. Chem. **3:** XXIV–XXV. 1906–1907.

Schulze, E. ('91). Ueber das Verhalten der Lupinenkeimlinge gegen destillirtes Wasser. Landw. Jahrb. **20:** p. 236. 1891.

Sjöqvist, J. ('95.) Physiologisch-chemische Beobachtungen über Salzsäure. Skand. Archiv f. Physiol. **5:** 277–376. *pl. 7–8.* 1895.

Stiles, W., and Jörgensen, I. ('14). The measurement of electrical conductivity as a method of investigation in plant physiology. New Phytol. **13:** 226-242. *f. 1–5.* 1914.

True, R. H. ('14). The harmful action of distilled water. Am. Jour. Bot. **1:** 255–273. *f. 1.* 1914.

————, and Bartlett, H. H. ('12). Absorption and excretion of salts by roots, as influenced by concentration and composition of culture solutions. I. Concentration relations of dilute solutions of calcium and magnesium nitrates to pea roots. U. S. Dept. Agr., Bur. Pl. Ind., Bul. 231: 1–36. *pl. 1 f. 1–21.* 1912.

————, ————, ('15). The exchange of ions between the roots of Lupinus albus and culture solutions containing one nutrient salt. Am. Jour. Bot. **2:** 255–278. *f. 1–13.* 1915.

————, ————, ('15a). The exchange of ions between the roots of Lupinus albus and culture solutions containing two nutrient salts. *Ibid.* **2:** 311–323. *f. 1–3.* 1915.

Wächter, W. ('05). Untersuchungen über den Austritt von Zucker aus den Zellen der Speicherorgane von Allium Cepa und Beta vulgaris. Jahrb. f. wiss. Bot. **41:** 165–220. *1 f.* 1905.

Walker, J. ('10). Introduction to physical chemistry. London, 1910. [See p. 237 ff.]

Whitney, M., and Briggs, L. J. ('97). An electrical method of determining the temperature of soils. U. S. Dept. Agr., Div. Soils, Bul. 7: 1–15. *f. 1.* 1897.

————, Gardner, F. D., and Briggs, L. J. ('97). An electrical method of determining the moisture content of arable soils. *Ibid.* 6: 1–26. *f. 1–6.* 1897.

————, and Means, T. H. ('97). An electrical method of determining the soluble salt content of soils, with some results of investigations on the effect of water and soluble salts on the electrical resistance of soils. *Ibid.* 8: 1–30. *f. 1–6.* 1897.

Winckler, A. ('04). Ist destilliertes Wasser ein Gift? Zeitschr. diätet. u. phys. Therapie **8:** 567–571. 1904–1905.

Woodward, J. (1699). Some thoughts and experiments concerning vegetation. Roy. Soc. London, Phil. Trans. **21:** 193–227. 1699.

EXPLANATION OF PLATE

PLATE 13

Figure 1.

Culture no.		Conditions of growth.
1	(2)	*Unrenewed distilled H_2O, 45 days.
2	(3)	Renewed distilled H_2O, 45 days.
3	(6)	1 day dist. H_2O, 44 days in renewed full nutr.
4	(8)	2 days dist. H_2O, 43 days in renewed full nutr.
5	(10)	5 days dist. H_2O, 40 days in renewed full nutr.
6	(14)	10 days dist. H_2O (renewed), 35 in renewed full nutr.
7	(18)	15 days dist. H_2O (renewed), 30 in renewed full nutr.
8	(22)	20 days dist. H_2O (renewed), 25 in renewed full nutr.
9	(23)	45 days in unrenewed full nutr.
10	(25)	45 days in renewed full nutr.

Figure 2.

1	(9)	5 days in unrenewed dist. H_2O, 40 days in unrenewed full nutr.
2	(10)	5 days in unrenewed dist. H_2O, 40 days in renewed full nutr.
3	(11)	10 days in unrenewed dist. H_2O, 35 days in unrenewed full nutr.
4	(12)	10 days in unrenewed dist. H_2O, 35 days in renewed full nutr.
5	(13)	10 days in renewed dist. H_2O, 35 days in unrenewed full nutr.
6	(14)	10 days in renewed dist. H_2O, 35 days in renewed full nutr.
7	(19)	20 days in unrenewed dist. H_2O, 25 days in unrenewed full nutr.
8	(20)	20 days in unrenewed dist. H_2O, 25 days in renewed full nutr.
9	(21)	20 days in renewed dist. H_2O, 25 days in unrenewed full nutr.
10	(22)	20 days in renewed dist. H_2O, 25 days in renewed full nutr.

* The numbers in parentheses correspond to the culture numbers of series 1. (See table I.)

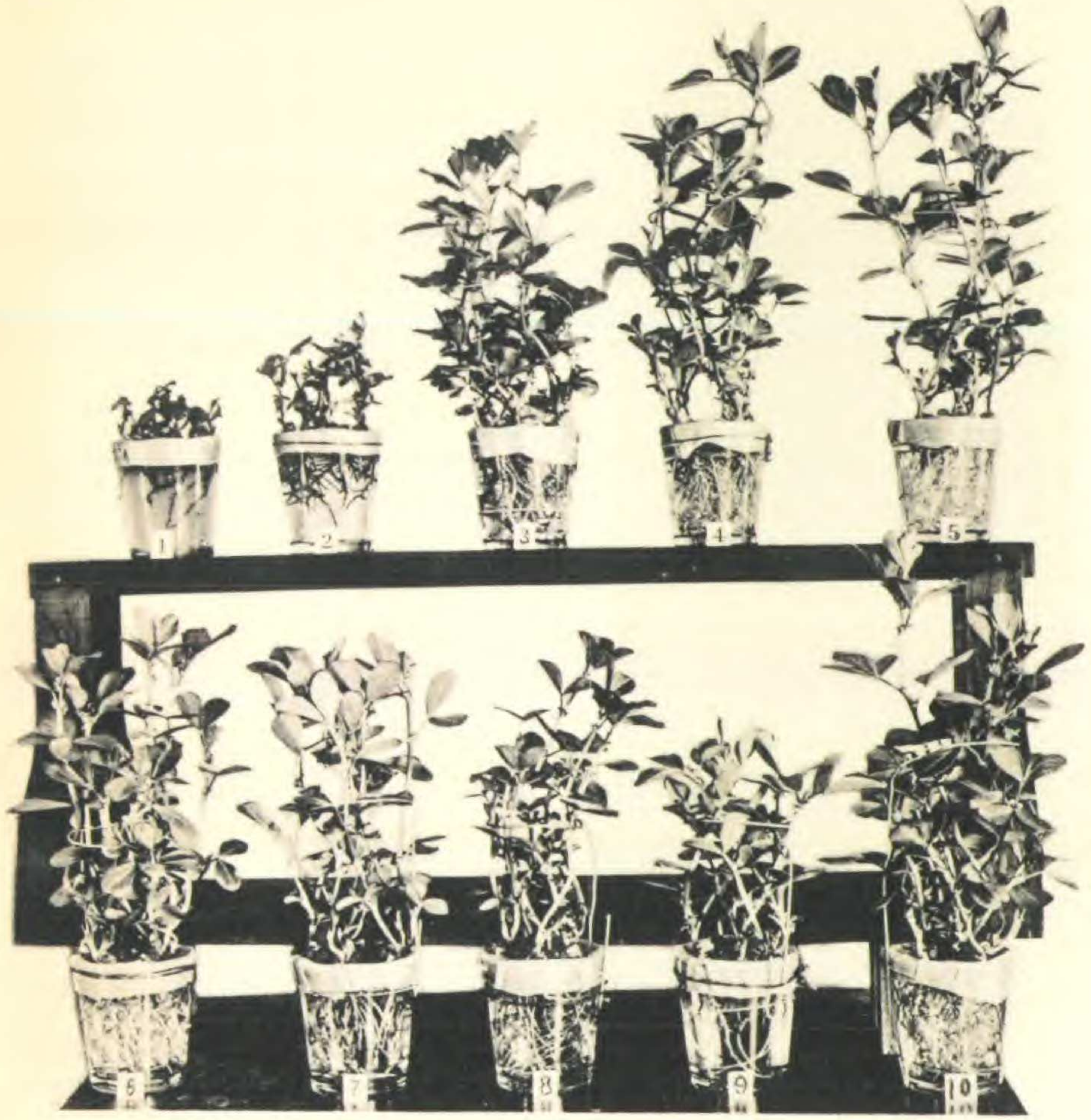

Fig. 1

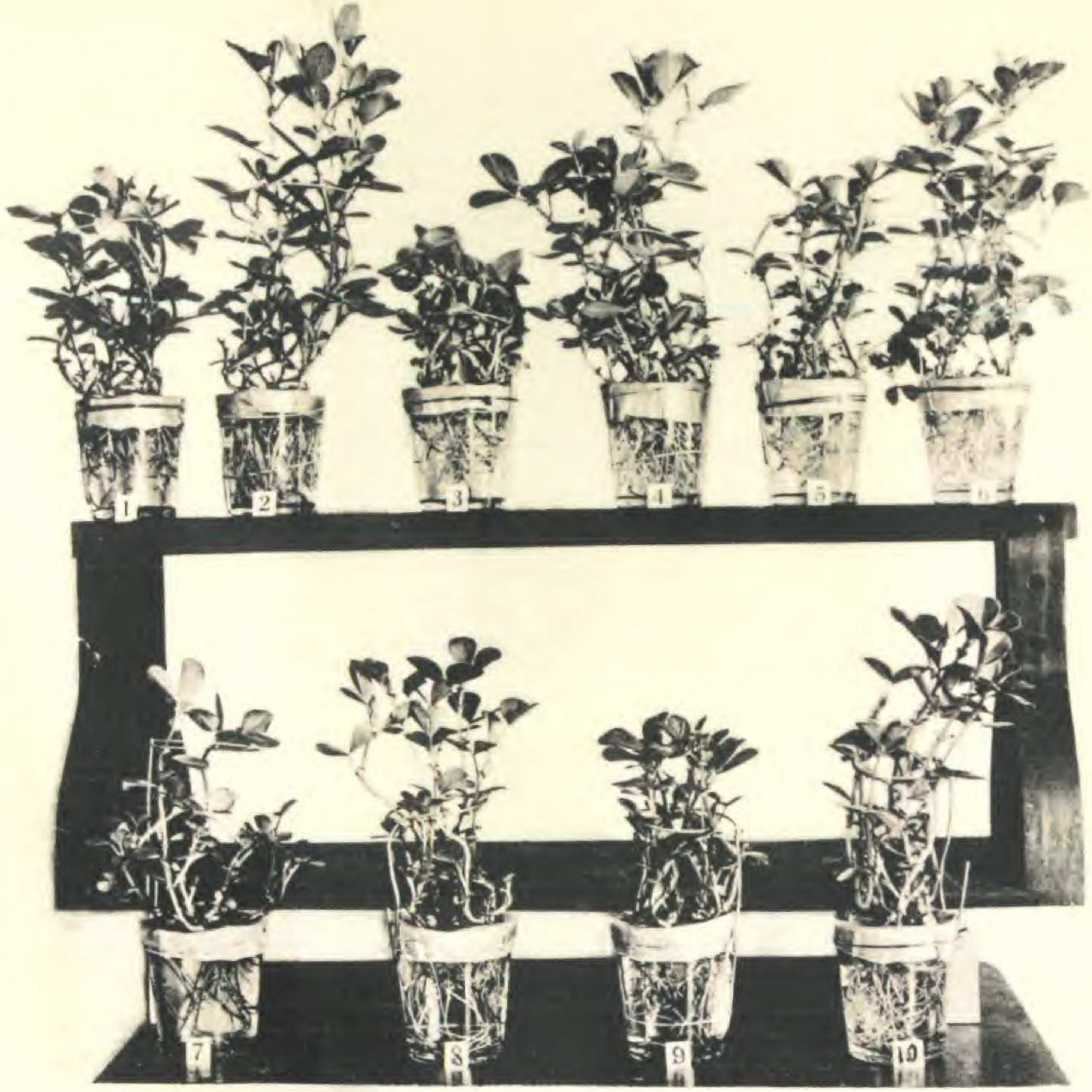

Fig. 2

MERRILL—DISTILLED WATER

COCKAYNE, BOSTON

Explanation of Plate

PLATE 14

Figure 1.

Culture no.		Conditions of growth.
1	(2)*	33 days in unrenewed dist. H_2O at time picture was taken.
2	(3)	33 days in renewed dist. H_2O at time picture was taken.
3	(6)	1 day in dist. H_2O, 32 days in renewed full nutr.
4	(8)	2 days in dist. H_2O, 31 days in renewed full nutr.
5	(10)	5 days in unrenewed dist. H_2O, 28 days in renewed full nutr.
6	(14)	10 days in renewed dist. H_2O, 23 days in renewed full nutr.
7	(18)	15 days in renewed dist. H_2O, 18 days in renewed full nutr.
8	(22)	20 days in renewed dist. H_2O, 13 days in renewed full nutr.
9	(23)	33 days in unrenewed full nutr.
10	(26)	33 days in renewed full nutr.
Figure 2.		
1	(9)	5 days in unrenewed dist. H_2O, 28 days in unrenewed full nutr.
2	(10)	5 days in unrenewed dist. H_2O, 28 days in renewed full nutr.
3	(11)	10 days in unrenewed dist. H_2O, 23 days in unrenewed full nutr.
4	(12)	10 days in unrenewed dist. H_2O, 23 days in renewed full nutr.
5	(13)	10 days in renewed dist. H_2O, 23 days in unrenewed full nutr.
6	(14)	10 days in renewed dist. H_2O, 23 days in renewed full nutr.
7	(19)	20 days in unrenewed dist. H_2O, 13 days in unrenewed full nutr.
8	(20)	20 days in unrenewed dist. H_2O, 13 days in renewed full nutr.
9	(21)	20 days in renewed dist. H_2O, 13 days in unrenewed full nutr.
10	(22)	20 days in renewed dist. H_2O, 13 days in renewed full nutr.

---

* The numbers in parentheses correspond to the culture numbers of series 2. (See table III.)

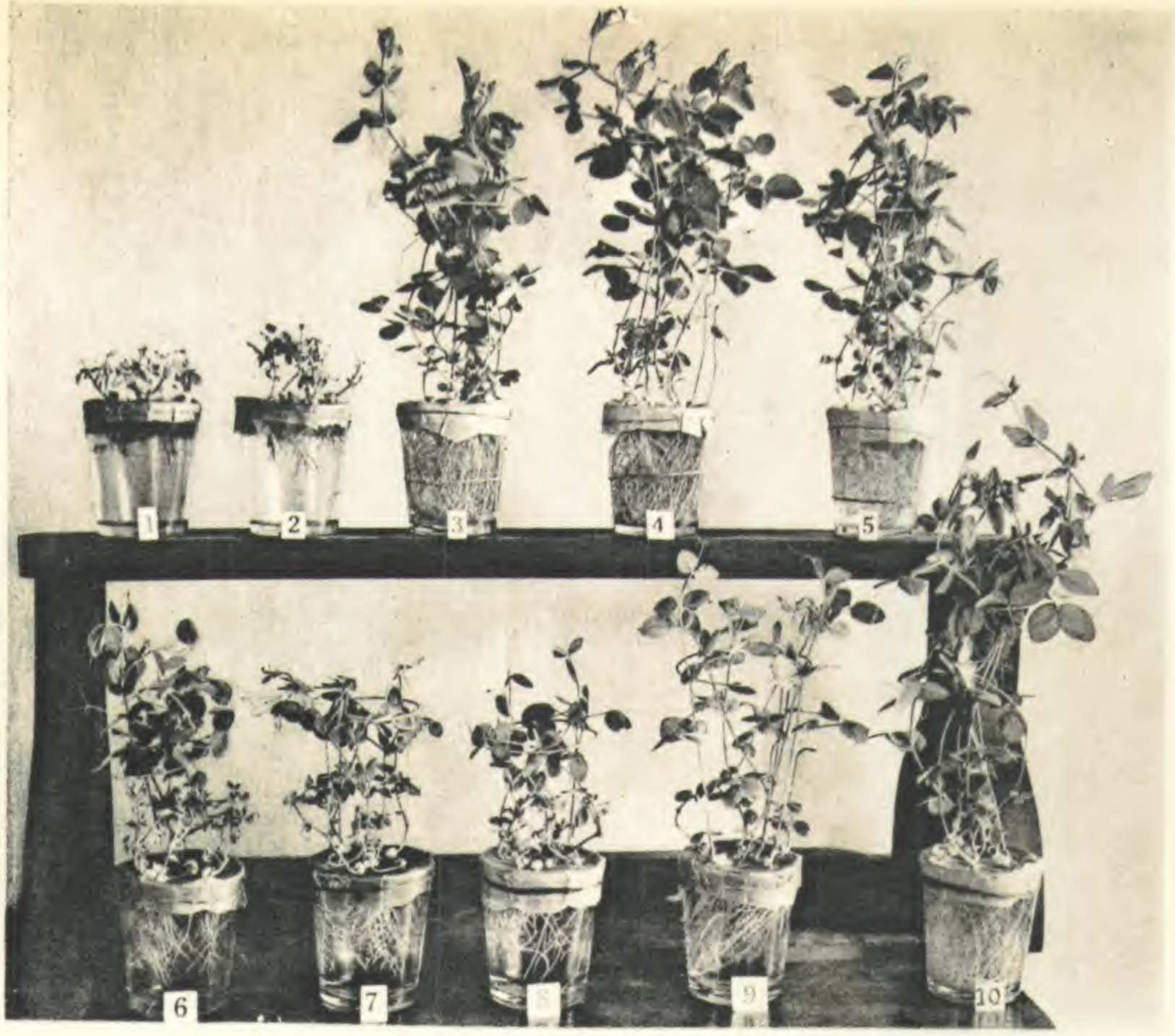

Fig. 1

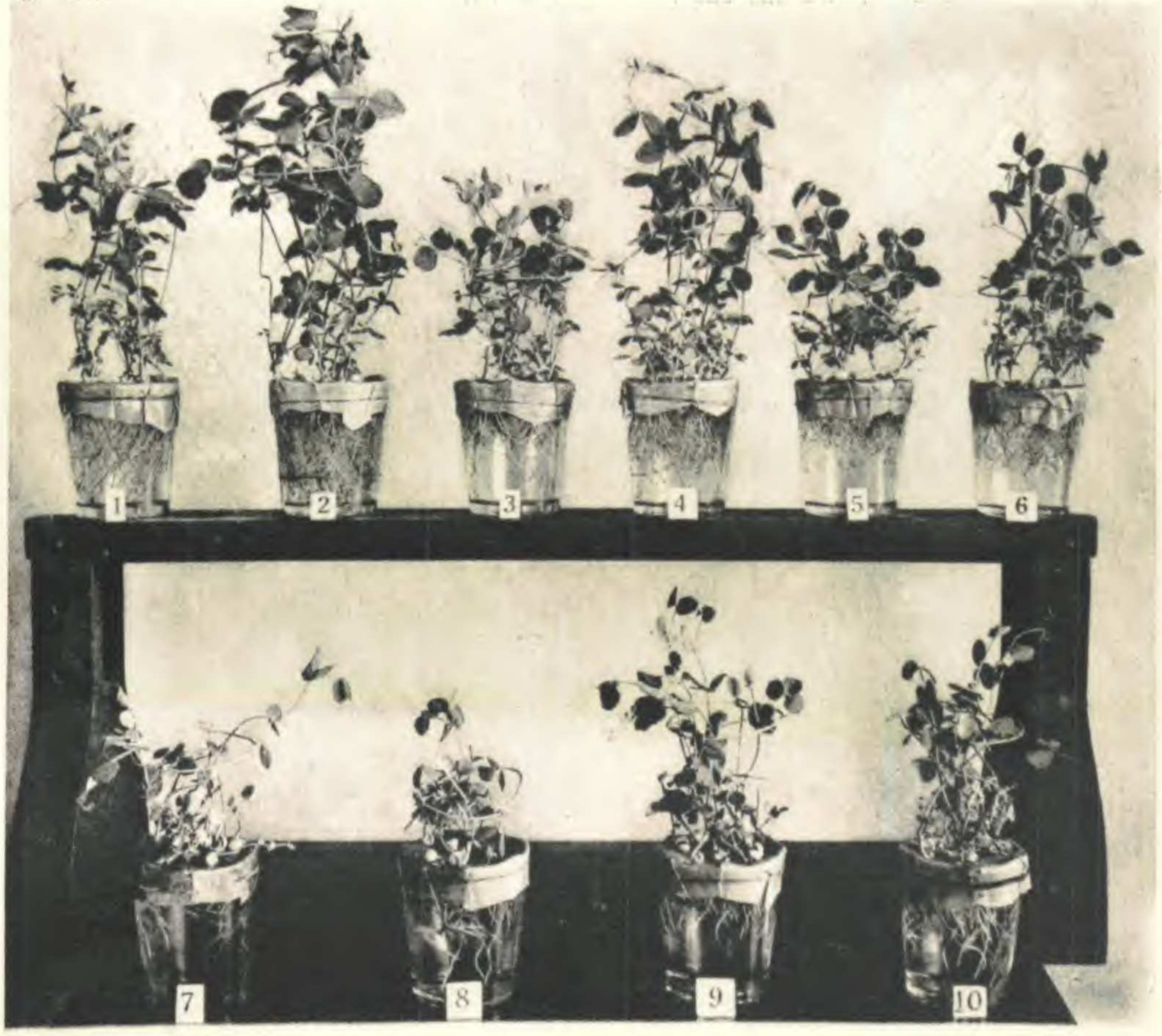

Fig. 2

MERRILL—DISTILLED WATER

COCKAYNE, BOSTON

EXPLANATION OF PLATE

PLATE 15

Figure 1. Culture no.		Conditions of growth.
1	(1)*	10 days in unrenewed dist. H_2O, 42 days in unrenewed full nutr.
2	(2)	10 days in unrenewed dist. H_2O, 42 days in renewed full nutr.
3	(3)	10 days in renewed dist. H_2O, 42 days in unrenewed full nutr.
4	(4)	10 days in renewed dist. H_2O, 42 days in renewed full nutr.
5	(5)	20 days in unrenewed dist. H_2O, 32 days in unrenewed full nutr.
6	(6)	20 days in unrenewed dist. H_2O, 32 days in renewed full nutr.
7	(7)	20 days in renewed dist. H_2O, 32 days in unrenewed full nutr.
8	(8)	20 days in renewed dist. H_2O, 32 days in renewed full nutr.
9	(9)	30 days in unrenewed dist. H_2O, 22 days in unrenewed full nutr.
10	(10)	30 days in unrenewed dist. H_2O, 22 days in renewed full nutr.
11	(11)	30 days in renewed dist. H_2O, 22 days in unrenewed full nutr.
12	(12)	30 days in renewed dist. H_2O, 22 days in renewed full nutr.
13	(13)	40 days in unrenewed dist. H_2O, 12 days in unrenewed full nutr.
14	(15)	40 days in renewed dist. H_2O, 12 days in unrenewed full nutr.
Figure 2.		
1	(1)†	32 days in unrenewed dist. H_2O.
2	(2)	32 days in renewed dist. H_2O.
3	(20)	32 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$.
4	(22)	1 day in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 31 days unrenewed full nutr.
5	(23)	2 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 30 days unnewed full nutr.
6	(24)	4 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 28 days unnewed full nutr.
7	(25)	8 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 24 days unrenewed full nutr.
8	(26)	12 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 20 days unnewed full nutr.
9	(27)	16 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 16 days unrenewed full nutr.
10	(28)	20 days in unrenewed N/20 $MgCl_2$ & N/1000 $CaCl_2$, 12 days unrenewed full nutr.
11	(29)	32 days in unrenewed full nutrient solution.

*The numbers in parentheses correspond to the culture numbers of series 3. (See table V.)

†The numbers in parentheses correspond to the culture numbers of series 4. (See table VI.)

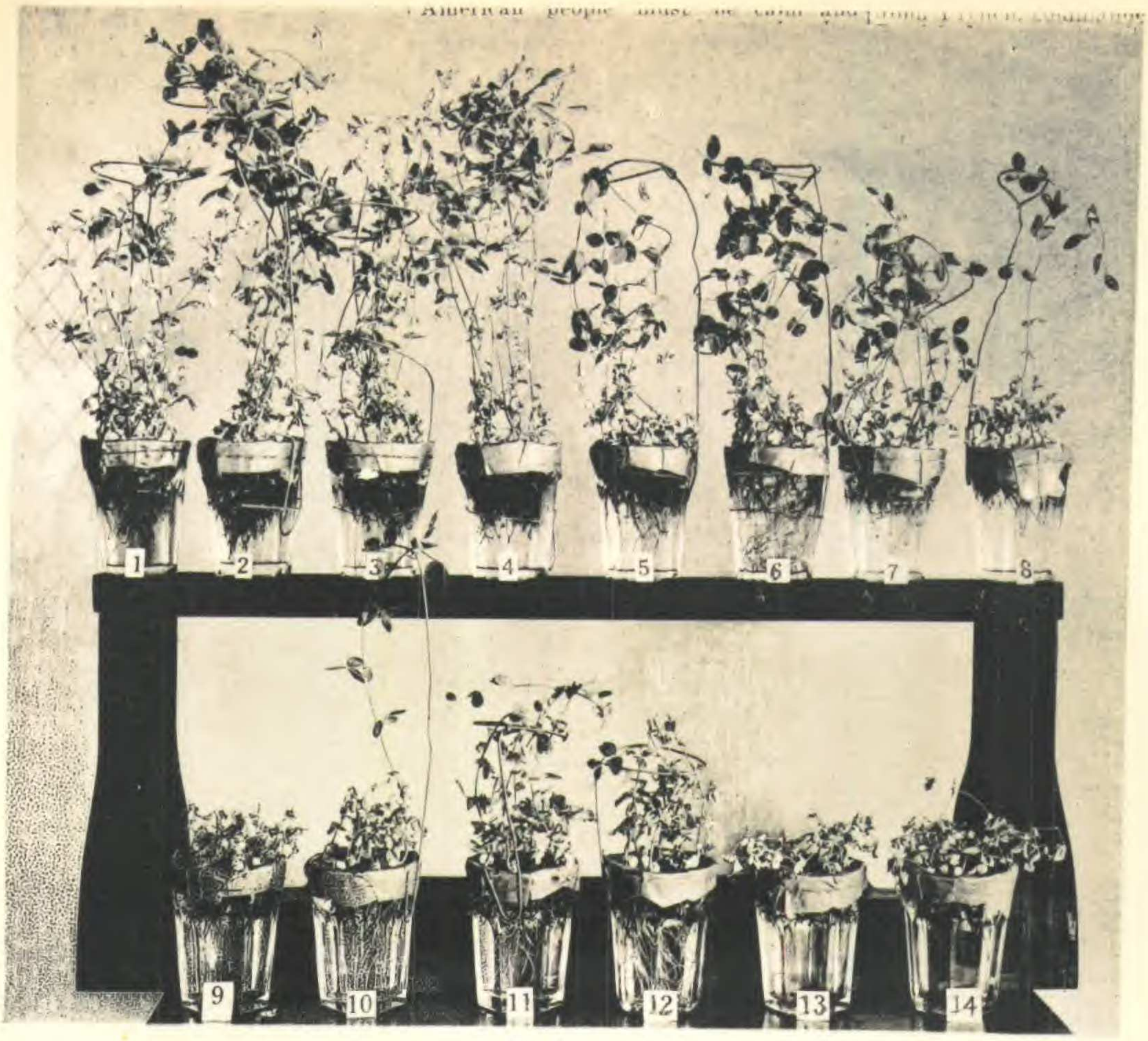

Fig. 1

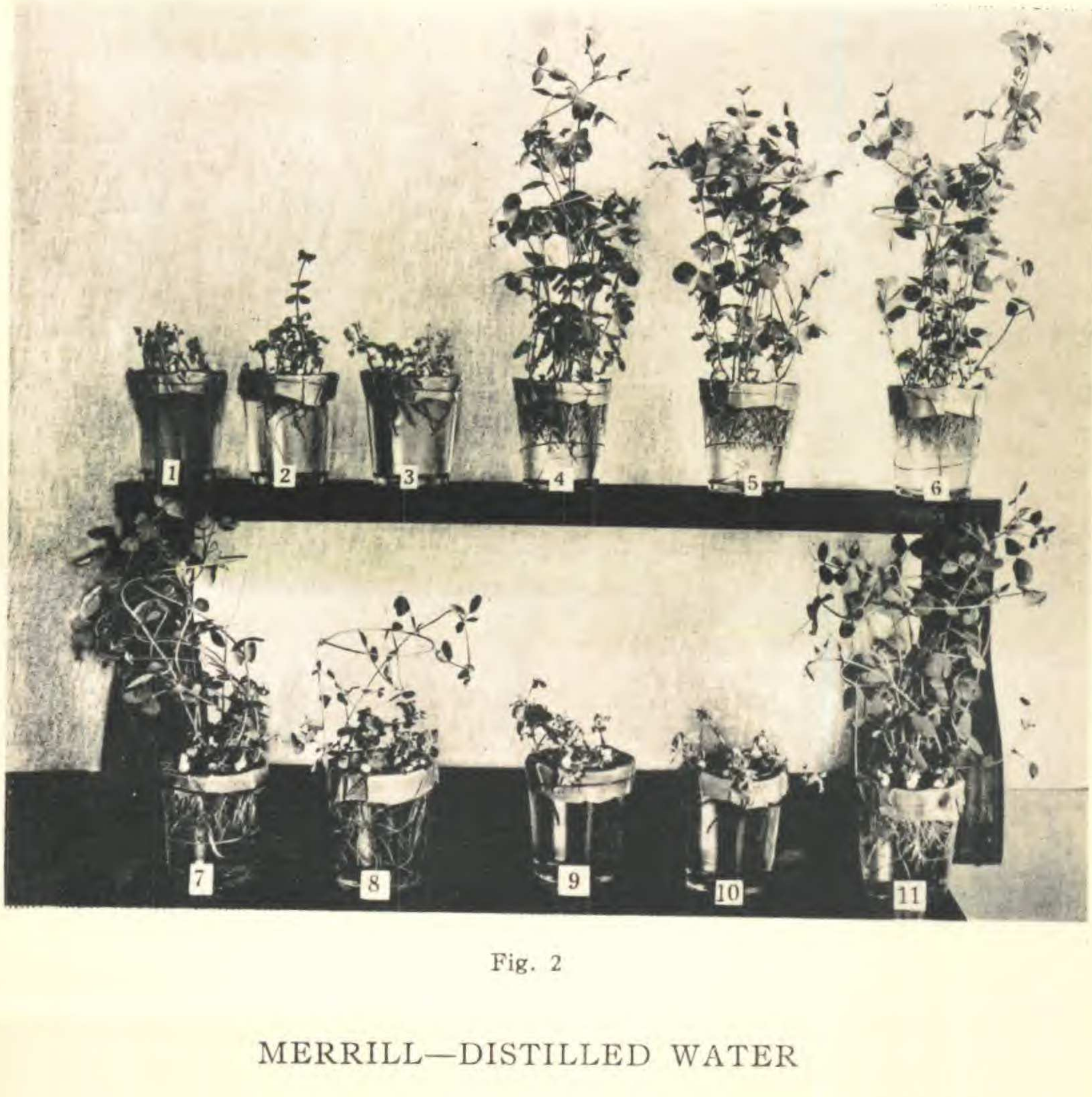

Fig. 2

MERRILL—DISTILLED WATER

COCKAYNE, BOSTON

Explanation of Plate

PLATE 16

Figure 1.

Culture no.	Conditions of growth.
1	(1)*30 days in unrenewed distilled H_2O.
2	(2) 30 days in renewed distilled H_2O.
3	(3) 30 days in distilled H_2O, sterilized every four days.
4	(4) 30 days in distilled H_2O, sterilized every four days.
5	(5) 30 days in unrenewed full nutrient solution.
6	(6) 30 days in renewed full nutrient solution.

Figure 2.

Showing the method used for seed germination.

*The numbers in parentheses also correspond to the culture numbers of series 5. (See table VII.)

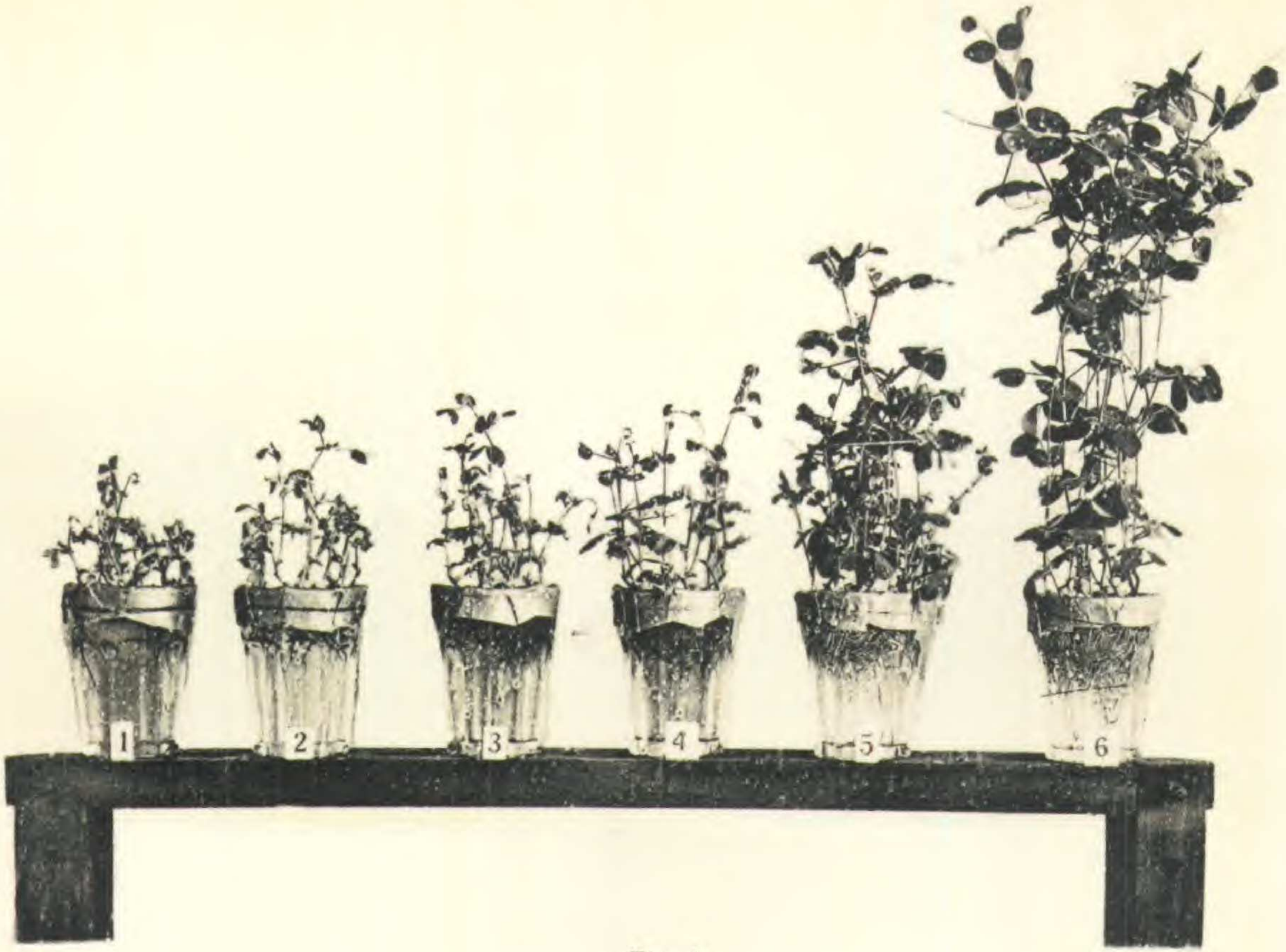

Fig. 1

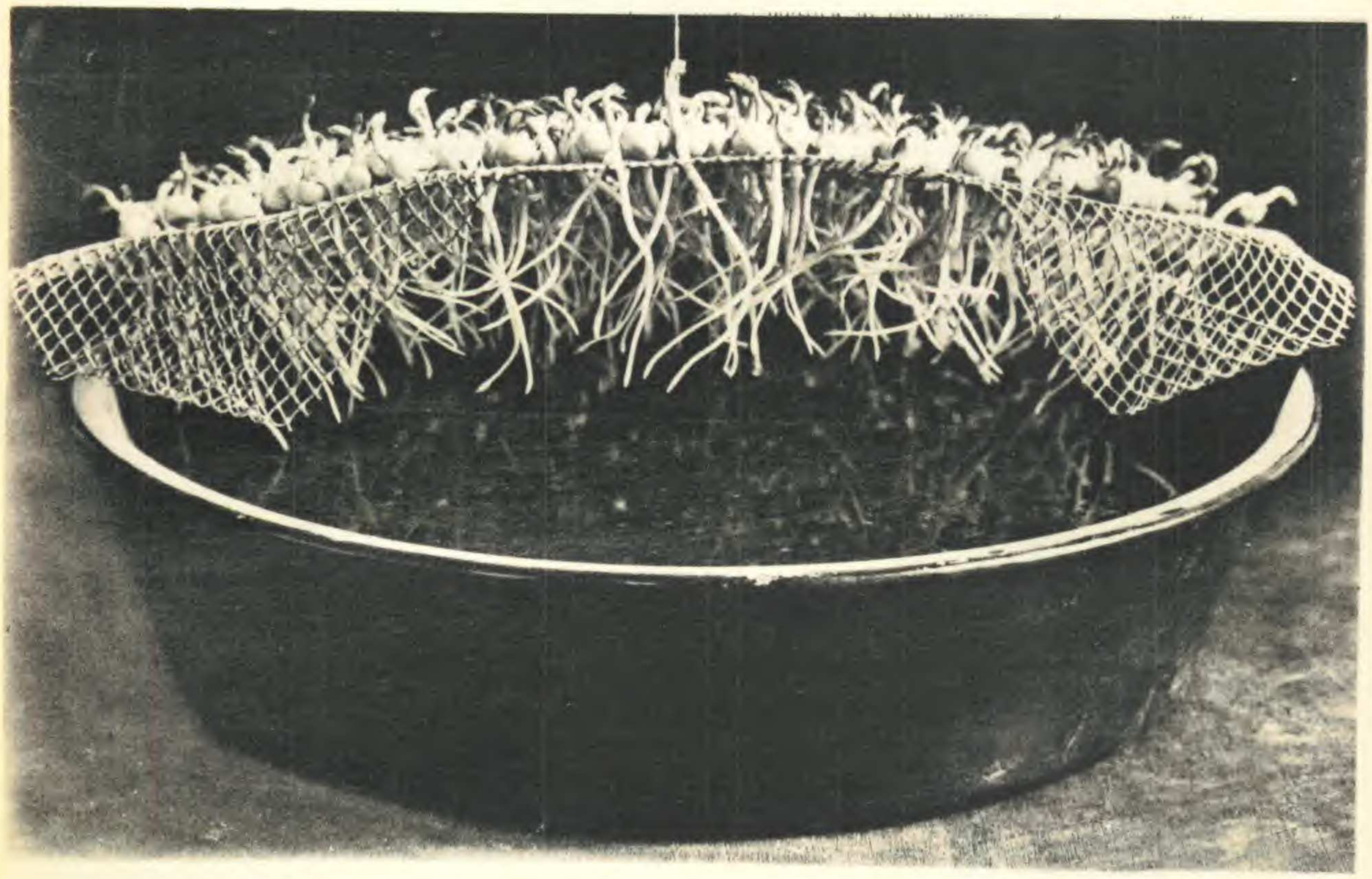

Fig. 2

MERRILL—DISTILLED WATER

COCKAYNE, BOSTON